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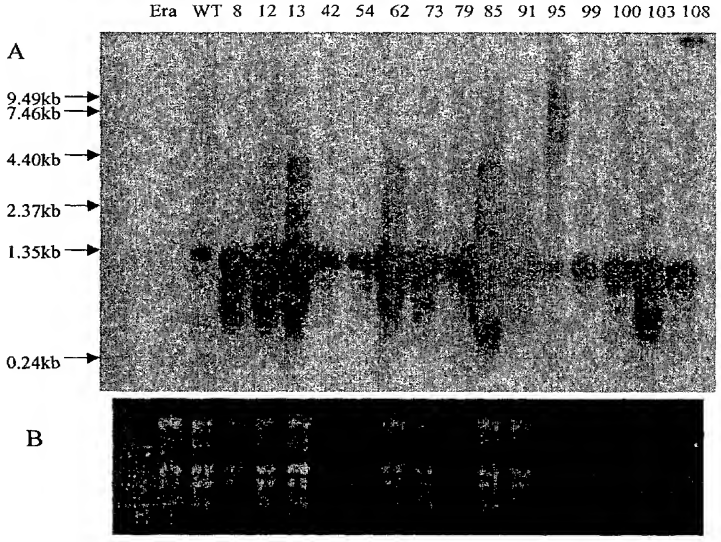

Northern blot of ΔN90AtFTB arabidopsis plants

A. Northern blot probed with ΔN90AtFTB DNA probe

B. Ethidium bromide stain of agarose gel showing RNA loading per lane

(57) Abstract: The novel constructs and methods of this invention improve tolerance in plants to environmental stresses and senescence. Nucleic acids encoding a plant farnesyl transferase are described, as are transgenic plants and seeds incorporating these nucleic acids and proteins. Also provided are inhibitors of naturally-occurring farnesyl transferase which, when expressed, will enhance drought tolerance in the plants, improve resistance to senescence and modify growth habit.

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STRESS TOLERANCE AND DELAYED SENESCENCE IN PLANTS

BACKGROUND OF THE INVENTION

Most higher plants encounter at least transient decreases in relative water content at some stage of their life cycle and, as a result, have evolved a number of desiccation protection mechanisms. If however, the change in water deficit is prolonged the effects on the plant's growth and development can be profound. Decreased water content due to drought, cold or salt stresses can irreparably damage plant cells which in turn limits plant growth and crop productivity in agriculture.

Plants respond to adverse conditions of drought, salinity and cold with a variety of morphological and physiological changes. Although our understanding of plant tolerance mechanisms to these stresses is fragmentary, the plant hormone abscisic acid (ABA) has been proposed to be an essential mediator between environmental stimulus and plant responses. ABA levels increase in response to water deficits and exogenously applied ABA mimics many of the responses normally induced by water stress. Once ABA is synthesized it causes the closure of the leaf stomata thereby decreasing water loss through transpiration.

The identification of genes that transduce ABA into a cellular response opens the possibility of exploiting these regulators to enhance desiccation tolerance in crop species. In principle, these ABA signalling genes can be coupled with the appropriate controlling elements to allow optimal plant growth and development. Thus, not only would these genes allow the genetic tailoring of crops to withstand transitory environmental insults, they should also broaden the environments where traditional crops can be grown.

In addition, little is known of the genetic mechanisms which control plant growth and development. Genes which further affect other metabolic processes such as senescence and growth habits of plants can be useful in a wide variety of crop and horticultural plants.

SUMMARY OF THE INVENTION

This invention relates to isolated nucleic acids which encode a farnesyl transferase comprising SEQ ID NO:1 or SEQ ID NO:172. Nucleic acids also encompassed by this invention are such hybridizing sequences which encode the functional equivalent or fragement

thereof of SEQ ID NO:1 or SEQ ID NO:172. The present invention also relates to a method for enhancing the drought tolerance of plants using inhibitors of the products encoded by these nucleic acids. Further, this invention relates to the control of regulatory functions in photosynthetic organisms; for example, in the control of growth habit, flowering, seed production, seed germination, and senescence in such organisms.

This invention also relates to a method for enhancing the drought or stress tolerance of plants by means of alterations in isolated or recombinant nucleic acids encoding a farnesyl transferase (Ftase) protein or fragment thereof or its functional equivalent. Nucleic acids which hybridize to the Ftase-encoding gene (ERA1) are also encompassed by this invention when such hybridizing sequences encode the functional equivalent of the Ftase protein. The present invention also relates to a method for enhancing the drought tolerance of plants through the genetic manipulation of ERA1 gene and its functional equivalents to improve stress tolerance in crop plants. Loss of ERA1 gene function confers enhanced tolerance to drought at the level of the mature plant. The nature of an *era*1 mutant with loss of Ftase activity, for example, demonstrates that inhibition of farnesylation enhances ABA responses in a plant.

Further, this invention relates to inhibition of senescence in photosynthetic organisms through inhibition of farnesyl transferase activity. The resulting photosynthetic organisms stay green and tissue viability is maintained for a longer period of time. Thus, methods to provide greener plants and a reduction in senescence are part of this invention.

In yet another embodiment, methods are provided to modify the growth habit and flower induction of plants. Loss of ERA1 gene function under particular environmental conditions results in a reduction in the number of lateral branches produced on a plant and an increase in the number of flowers per inflorescence.

The invention also provides method of producing a transgenic plant, which has an altered phenotype such as increased tolerance to stress (e.g., water deficit, increased biomass, increased yield), delayed senescence or increased ABA sensitivity by introducing into a plant cell a compound that inhibits farnesylation of a polypeptide comprising a CaaX motif. By inhibit Farnesylation is meant to include that the compound inhibits one or more steps in the three step process of farnesylation. In one aspect the compound inhibits farnesyltransferase, prenylprotease or prenylcysteine carboxyl methytransferase expression or activity. Alternatively, the compound is a anti-sense farnesyl transferase nucleic acid or a farnesyl transferase double stranded RNA-inhibition hair pin nucleic acid. In some aspects the nucleic acid is operably linked to a promoter such as a constitutive promoter, an ABA inducible promoter, tissue specific promoters or a guard cell-specific promoter.

Exemplary anti-antisense nucleic acids are 20 or more consecutive nucleic acids complementary to SEQ ID NO: 1, 14, 40, 43, 80-85 or 172. Alternatively the anti-sense nucleic acids includes SEQ ID NO: 36, 41, 44 or 54-64.

In various aspects the compound is a nucleic acid encoding a farnesyltransferase, prenylprotease or prenylcysteine carboxyl methytransferase polypeptide of fragment thereof. Alternatively, the compound is a nucleic acid encoding a mutated farnesyltransferase, prenylprotease or prenylcysteine carboxyl methytransferase polypeptide of fragment thereof. By mutated is meant that the polypeptide lacks at least on activity of the wild type polypeptide such as for example, subunit interaction, substrate binding or enzyme catalysis. A mutated polypeptide forms a dimer, such as a heterodimer. For example, a mutated farnesytransferase beta polypeptide forms a dimer with a farnesyltransferase alpha polypeptide. Preferably, the polypeptide is less than 400, 350, 314 , 300 or 200 amino acids in length. For example, the compound includes SEQ ID NO: 1, 14, 40, 43, 80-85 or 172.

In a further aspect the compound is a nucleic acid encoding a CaaX motif or a nucleic acid encoding a CaaX motif operably linked to a promoter.

Also included in the invention are the plants produced by the methods of the invention and the seed produced by the plants which produce a plant that has an altered phenotype.

This invention also relates to a regulatory sequence useful for genetic engineering of plant cells to provide a method of controlling the tissue pattern of expression of DNA sequences linked to this novel regulatory sequence.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1C show the nucleic acid sequence of the ERA1 gene (SEQ ID NO:1) in which the introns are underlined and the start codon (ATG) is at nucleotide positions 1-3.

Figure 2 is the amino acid sequence of the ERA1 protein (SEQ ID NO:2).

Figures 3A-3B show the nucleic acid sequence of the ERA1 promoter (SEQ ID NO:3).

Figure 4 is the amino acid sequence of the β subunit farnesylation domain from *Arabidopsis* (Arab.) (SEQ ID NO:2) aligned with the β subunit farnesylation domains from pea (SEQ ID NO:4), yeast (SEQ ID NO:5) and rat (SEQ ID NO:6). Residues that are identical to the *Arabidopsis* sequence are indicated with a dot. A dash indicates a blank. The amino acid positions of the *Arabidopsis* gene are indicated on the right-hand side.

Figure 5 is a photograph of an *era*1-transformed *Arabidopsis* plant (right) compared to the wild-type (control; *i.e.*, naturally-occurring) plant (left) under extremely dry conditions.

Figure 6 is a graph comparing the water content of *Arabidopsis* plants with inactivated or mutant Ftase activity (*M. Columbia*, *era* 1-2) and controls (M.C. control, *era* 1-2 control).

Figure 7 is a graph comparing the rate of water loss for the *Arabidopsis* plants with inactivated or mutant Ftase activity (*M. Columbia*, *era* 1-2) and controls (M.C. control, *era* 1-2 control).

Figures 8A-8E are comparisons of aging leaves from control (wild-type) and *era*-2 mutant plants.

Figures 9A-9C are comparisons of transcript levels in aging leaves from control (wild-type) and *era*-2 mutant plants.

Figure 10 is an illustration depicting the pBI121 antisense FTA vector construct.

Figure 11 is an illustration of genomic Southern hybridization analysis of anti-FTA transgenic *Arabidopsis thaliana*.

Figure 12 is an illustration of Northern analysis of five 35S-anti-FTA *Arabidopsis thaliana* lines (T3 plants).

Figure 13 shows a Western expression analysis using anti-FTA antibodies to detect the FTA polypeptides.

Figure 14 is a set of photographs showing ABA effects on seedling growth and development. FTA antisense transgenic seedlings exhibit enhanced ABA sensitivity.

Figure 15 shows the effect of ABA on seedling growth and development.

Figure 16 shows photographs of wild type Columbia (A) and four antisense FTA transgenic lines (B, C, D, E) of *Arabidopsis thaliana* after 8 days without watering.

Figure 17 is an illustration of the homology among FTA nucleic acid (A) and amino acid (B) sequences from various plant species based on ClustalW anaysis (percent identity shown).

Figure 18 is an illustration of the homology among FTB nucleic acid and amino acid sequences from various plant species based on ClustalW anaysis (percent identity shown).

Figure 19 is an illustration of transgenic performance during water stress.

Figure 20 is an illustration of shoot fresh weight, or biomass accumulation, after 6 days of water stress treatment and 6 days recovery time.

Figure 21 is an illustration of seed yield (grams) obtained under optimal conditions or following a 6 day water stress treatment.

Figure 22 is an illustration of vegetative growth under optimal conditions, shown is shoot fresh weight 6 days after the first flower opened.

Figure 23 is an illustration of the effect of a biotic stress coupled with drought stress treatment on seed yield.

Figure 24 is a representative illustration of gel electrophoresis analysis of PCR products in an assay to detect transgenic lines of *Brassica napus*.

Figure 25. is a schematic representation of the vector constructs; A) pBI121-AtCPP, B) pBI121-antisense-AtCPP, C) pBI121-HP-AtCPP.

Figure 26. is an illustration of (A) nucleic acid and (B) amino acid sequence identities as determined by ClustalW analysis.

Figure 27. is a scan of a typical Southern blot of transgenic *Arabidopsis* T1 lines carrying the pBI121-AtCPP construct.

Figure 28. is a scan of a typical Southern blot of transgenic *Arabidopsis* T3 lines carrying the pBI121-HP-AtCPP construct.

Figure 29. is a scan of a typical Southern blot of transgenic *Arabidopsis* lines carrying the pRD29A-AtCPP construct.

Figure 30. is a scan of a typical Southern blot of transgenic *Arabidopsis* lines carrying the pRD29A-HP-AtCPP construct.

Figure 31 is an illustration showing the relative expression of AtCPP mRNA transcript (solid bars) and AtCPP protein levels (stippled bars) in several pBI121-AtCPP transgenic lines.

Figure 32. is a histogram showing the percentage of lines which were categorized as ABA sensitive, moderately ABA sensitive or ABA insensitive. Seedlings were assessed on agar plates containing 1 μM ABA and scored at 21 days growth. Thirty-six lines of the pBI121-AtCPP over-expression construct were assessed at 21 days by leaf and seedling development. Thirty-two lines of the 35S-HP-AtCPP down-regulation construct were assessed at 21 days for leaf and seedling development. Each line was assessed by plating approximately 100 seeds per plate and the seedlings scored and recorded as the percent insensitive seedlings per plate. Each line was then expressed as a percent of wild type (Wt). Lines were categorized as sensitive (less than 1% of Wt) solid bars, intermediate (1-50% of Wt) diagonally lined or insensitive (greater than 50% of Wt) stippled, based on their relationship to Wt and the percentage of each category plotted as a histogram.

Figure 33. is an illustration showing the response of wild type and a pRD29A-HP-AtCPP transgenic line to various concentrations of ABA in two week old seedlings.

Figure 34. is a histogram showing the analysis of transgenic plants containing the pBI121-AtCPP over-expression construct, (SEQ ID NO:4). Water loss per gram shoot dry weight after four days of water stress treatment. Lines that are marked with a star are those which were strongly ABA sensitive. Lines marked with a triangle are moderately ABA sensitive. Bars represent means of eight replicates. Lines marked with a filled dot above the bar represents lines which were significantly different from control at a p=0.05 value.

Figure 35. is a histogram showing seed yield in grams of transgenic *Arabidopsis* lines of pBI121-AtCPP grown under optimal water conditions

Figure 36. is a bar chart howing growth and yield of transgenic *Arabidopsis* lines of pBI121-AtCPP grown under optimal watering conditions plus a biotic stress condition. Tields as a % of wild type, rosette leaf number, rosette leaf fresh weight and shoot dry weight are plotted.

Figure 37. are photographs showing rowth of transgenic *Arabidopsis* lines of pBI121-AtCPP grown on agar plates. Changes to root growth visible.

Figure 38. is a bar chart showing rowth of transgenic *Arabidopsis* lines of pRD29A-HP-AtCPP grown under optimal watering conditions. Rosette leaf number, rosette leaf dry weight and shoot dry weight are plotted.

Figure 39 . is an photograph showing Northern blot of ΔN90AtFTB arabidopsis plants

The foregoing and other objects, features and advantages of the invention will be apparent from the following more particular description of preferred embodiments of the invention, as illustrated in the accompanying drawings in which like reference characters refer to the same parts throughout the different views. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating the principles of the invention.

DETAILED DESCRIPTION OF THE INVENTION

This invention relates to transgenic plants that display an altered phenotype, e.g., increased tolerance to stress, delayed senescence, increased ABA sensitivity, increased yield, increased productivity and increased biomass and methods of producing the plants by introducing to a plant cell a compound that inhibits farnesylation of a polypeptide comprising a CaaX motif

Protein farnesylation, the addition of a C-terminal, 15 carbon chain to protein and subsequent processing is a three step enzymatic reaction including farnesylation, proteolytic cleavage and methylation. First, a farnesyltransferase adds the C-terminal 15 carbon chain to a conserved cysteine residue of the CaaX terminal motif, where "C" is a Cystine, "a" is an aliphatic amino acid and "X" is any amino acid. Second, the last three amino acid residues (aaX) are cleaved by a prenyl protease. Lastly, the modified cysteine is methylated by a methylase to create the final active product of the protein farnesylation pathway. The Applicant's have shown that over expression and down-regulation of the alpha or the beta farnesyl transferase gene in plant cells (i.e, the first step in farnesylation) results in plants with an altered phenotype such as but not limited to drought tolerance and delayed senescence. Applicants have also shown that over expression and down-regulation of the prenyl protease gene (i.e, the second step in farnesylation) in plant cells also results in a plant displaying an altered phenotype including for example but not limited to drought tolerance and increased resistance to biotic and abiotic stress. These results taken together support the hypothesis that modification of the expression of any of the enzymes in the farnesylation pathway (farnesytransferase, prenylprotease or prenycysteine carboxyl methytransferase in a plant cell will result in a plant displaying an altered phenotype

The present invention also provides novel farnesytransferase (*i.e.,* alpha and beta), (Ftase) and CaaX prenyl protease (CPP) nucleic acid sequences isolated from for example *Arabidopsis thaliana* (At) *Brassica napus* (Bn) and *Glycine Max* (Gm). The invention also provides farnesytransferase and CaaX prenyl protease antisense nucleic acids and constructs comprising these nucleic acids. The sequences are collectively referred to as "PPI nucleic acids", PPI polynucleotides" or "PPI antisense nucleic acids" and the corresponding encoded polypeptide is referred to as a "PPI polypeptide" or "PPI protein". Unless indicated otherwise, "PPI" is meant to refer to any of the novel sequences disclosed herein. Table A below summarizes the nucleic acids and polypeptides according to the invention

TABLE A

PPI Sequence Description	SEQ ID NO:
era1 (FTB)	1
era1 (FTB)	2
ERa1 promoter	3
FTB pea	4
FTB yeast	5
FTB rat	6
At FTA	7

At FTA	8
At FTA	9
pBI121-35S-anti-AtFTA	10
At FTA	11
Bn FTA	12
Bn FTA	13
Bn FTB	14
Bn FTB	15
primer	16
primer	17
primer	18
primer	19
primer	20
primer	21
primer	22
primer	23
primer	24
primer	25
primer	26
primer	27
primer	28
primer	29
primer	30
primer	31
primer	32
primer	33
primer	34
Bn FTA	35
Bn FTB	36
G max FTA	37
G max FTA	38
G max FTA	39
G max FTB	40
G max FTB	41
G max FTB	42
Zea maize FTB	43
Zea maize FTB	44
Zea maize FTB	45
pBI121-35S-AtFTA	46
pBI121-rd29A-anti-AtFTA	47
pBI121-35S-DA-AtFTA	48
pBI121-RD29A-DA-AtFTA	49
MuA-anti-GmFTA	50
RD29A-anti-GmFTA	51

MuA-HP-GmFTA-Nos-Term	52
RD29AP-HP-GmFTA-Nos-Term	53
pBI121-35S-Anti-AtFTB	54
pBI121-RD29AP-Anti-AtFTB	55
pBI121-35S-HP-AtFTB	56
pBI121-RD29AP-HP-AtFTB	57
pBI121-35S-AtFTB	58
MuA-anti-GmFTB-Nos-Term	59
RD29AP-anti-GmFTB-Nos-Term	60
MuA-HP-GmFTB-Nos-Term	61
RD29AP-HP-GmFTB-Nos-Term	62
MuA-anti-Zea maizeFTB-Nos-Term	63
MuA-HP-Zea maizeFTB-Nos-Term	64
Pea-FT-A	65
Tomato-FTA	66
Rice-FT-A	67
Zea mays-FT-A	68
Soy1-Ft-A	69
Soy2-FT-A	70
Triticum-FT-A	71
Pea-FT-A	72
Tomato-FTA	73
Rice-FT-A	74
Zea mays-FT-A	75
Soy1-Ft-A	76
Soy2-FT-A	77
Triticum-FT-A	78
N90AtFTB truncated FTB vector	79
Wiggum (FTB)	80
Dup-Soy-FTB	81
Dup-Corn-FTB	82
Pea-FT-B	83
Tomato-FTB	84

Tobacco-FTB	85
Primer SacI forward	86
Wiggum (FTB)	87
Dup-Soy-FTB	88
Dup-Corn-FTB	89
Pea-FT-B	90
Tomato-FTB	91
Tobacco-FTB	92
Consensus FTA	93
Consensus FTB	94
Consensus FTA	95
Consensus FTB	96
AtCPP	97
AtCPP	98
At-AFC1	
pBI121-AtCPP	99
pBI121-HP-AtCPP	100
AtCPP BamFW	101
AtCPP SmaRV	102
AtCPP-HP-SacFW	103
AtCPP-HP-SacRV	104
pBI121-AtCPP Forward	105
pBI121-antiAtCPP-SmaFW	106
pBI121-antiAtCPP-BamRV	107
p35S-HP-AtCPP Reverse	108
BnCPP	109
BnCPP	110
BnCPP antisense	111
GmCPP	112
GmCPP	113
GmCPP antisense	114
AtCPP antisense	115
BASF-AT1	116
BASF-AT1	117
BASF-AT2	118
BASF-AT2	119
BASF-Corn	120
BASF-Corn	121
BASF-Soy	122
BASF-Soy	123
AFC1	124
AFC1	125
AT4g01320	126
AT4g01320	127
AF007269	128
AF007269	129
pBI121-antisense-AtCPP	130
pRD29A-AtCPP	131
pRD29A-HP-AtCPP	132

pRD29A-antisense-AtCPP	133
MuA-AtCPP	134
MuA-GmCPP	135
pBI121-GmCPP	136
pBI121-HP-GmCPP	137
pBI121-antisense-GmCPP	138
pRD29A-GmCPP	139
pRD29A-HP-GmCPP	140
pRD29A-antisense-GmCPP	141
pBI121-BnCPP	142
pBI121-HP-BnCPP	143
pBI121-antisense-BnCPP	144
pRD29A-BnCPP	145
pRD29A-HP-BnCPP	146
pRD29A-antisense-BnCPP	147
MuA-BnCPP	148
GmCPP SmaFW	149
GmCPP SacRV	150
BnCPP-anti-SmaFW	151
BnCPP-anti-BamRV	152
BnCPP-HP-Sac-FW	153
BnCPP-HP-Sac-RV	154
BnCPP-HP-BamFW	155
BnCPP-HP-XbaRV	156
GmCPP-HP-Sac-FW	157
GmCPP-HP-Sac-RV	158
GmCPP-HP-BamFW	159
GmCPP-HP-XbaRV	160
pRD29AP	161
Nosterm-RV	162
Consensus- BASF	163
Consensus- BASF	164
Consensus- Generic	165
Consensus- Generic	166
Consensus- PPI	167
Consensus- PPI	168
Consensus- PPI/Generic	169
Consensus- PPI/Genreric	170
Primer BamHI REV	171
Full Length AtFTB	172
pBI121-AtFTB full length	173
pimer	174
primer	175
isoprenylcysteine carboxyl methyltransferase	176

Full Length AtFTB	177

This invention also relates to isolated nucleic acids and proteins encoded by these nucleic acids which modify the growth, reproduction and senescence of plants. In particular, the constructs of this invention include an isolated nucleic acid encoding a farnesyl transferase (Ftase) polypeptide comprising SEQ ID NO:1 or 172 or its functional equivalent or fragment thereof, and the Ftase polypeptides or proteins of fragments thereof encoded by these nucleic acids. In particular, this invention relates to a protein wherein the sequence is SEQ ID NO:2 or SEQ ID NO:177 .

Further included in this invention are nucleic acid constructs which comprise a promoter (ERA1 promoter) operably-linked to isolated nucleic acid comprising SEQ ID NO:1 or 172 or its functional equivalent or a complement of either. When incorporated into a plant, the ERA1 promoter is regulated in the guard cells of the plant and can affect water loss through the stomates. This promoter consists of a nucleic acid comprising SEQ ID NO:3 (Figure 3).

Transgenic plants, seeds, plant cell and tissues incorporating these constructs are also part of this invention. Accordingly, in one aspect of this invention, a method is provided for producing a gene product under the control of a promoter which operates primarily in guard cells through expression of a gene encoding the gene product in the cell of a plant comprising the steps of: transforming a plant cell with a DNA construct comprising a) a regulatory region comprising SEQ ID NO:3 or a functional portion thereof, DNA comprising a structural gene encoding a gene product, and a 3' untranslated region containing a polyadenylated region; regenerating a plant, photosynthetic organism or tissue culture from the cell; and placing the plant, photosynthetic organisms or tissue culture under conditions so that the promoter induces transcription of the structural gene and the gene product is expressed.

In the context of this disclosure, the terms "regulatory region" or "promoter" refer to a sequence of DNA, usually upstream (5') to the coding sequence of a structural gene, which controls the expression of the coding region by providing recognition and binding sites for RNA polymerase and/or other factors required for transcription to start at the correct site. The term "functional portion" or "functional fragment" refers to a truncated sequence of a promoter of this invention which maintains the capability of inducing transcription of an ERA structural gene under the conditions described for activity of an Ftase protein.

The constructs and methods described herein can be applied to all types of plants and other photosynthetic organisms, including, but not limited to: angiosperms (monocots and dicots), gymnosperms, spore-bearing or vegetatively-reproducing plants and the algae, including the cyanophyta (blue-green algae). Particularly preferred plants are those plants which provide commercially-valuable crops, such as corn, wheat, cotton, rice, canola, sugar cane, sugar beet, sunflowers, potatoes, tomatoes, broccoli, carrots, lettuce, apple, plum, orange, lemon, rose, and the like.

Further, the constructs and methods of this invention can be adapted to any plant part, protoplast, or tissue culture wherein the tissue is derived from a photosynthetic organism. The term "plant part" is meant to include a portion of a plant capable of producing a regenerated plant. Preferable plant parts include roots and shoots and meristematic portions thereof. Other plant parts encompassed by this invention are: leaves, flowers, seeds, epicotyls, hypocotyls, cotyledons, cotyledonary nodes, explants, pollen, ovules, meristematic or embryonic tissue, protoplasts, and the like. Transgenic plants can be regenerated from any of these plant parts, including tissue culture or protoplasts, and also from explants. Methods will vary according to the species of plant.

This invention relates to compositions and constructs comprising isolated nucleic acids (both DNA and RNA) encoding an Ftase and portions thereof of photosynthetic organisms. This invention further relates to compositions and constructs comprising isolated nucleic acids encoding an Ftase promoter. In particular, the ERA1 gene encoding the β subunit of Ftase from *Arabidopsis* and a regulatory sequence which regulates the transcription of the ERA1 gene have been isolated and sequenced. Nucleic acids which encode Ftases from photosynthetic organisms, and homologues or analogs of these nucleic acids, are encompassed by this invention.

The invention further relates to methods using isolated and/or recombinant nucleic acids (DNA or RNA) that are characterized by their ability to hybridize to (a) a nucleic acid encoding an Ftase protein or polypeptide, such as a nucleic acid having the sequences of SEQ ID NO:1 or 172 or (b) a portion of the foregoing (*e.g.*, a portion comprising the minimum nucleotides required to encode a functional Ftase protein; or by the ability to encode a polypeptide having the amino acid sequence of an Ftase (*e.g.*, SEQ ID NO:2 or SEQ ID NO:177 , or to encode functional equivalents thereof; *e.g.*, a polypeptide having at least 80% sequence similarity to SEQ ID NO:2 or SEQ ID NO:177 , which when incorporated into a plant cell, facilitates the growth habit, seed germination, and metabolism in a photosynthetic organism in the same manner as SEQ ID NO:1 or 172). A functional equivalent of an Ftase therefore, would have at

least an 80% similar amino acid sequence and similar characteristics to, or perform in substantially the same way as, the polypeptide encoded by SEQ ID NO:2 or SEQ ID NO:177 . A nucleic acid which hybridizes to a nucleic acid encoding an Ftase polypeptide such as SEQ ID NO:2 or SEQ ID NO:177 can be double- or single-stranded. Hybridization to DNA such as DNA having the sequence SEQ ID NO:1 or 172, includes hybridization to the strand shown or its complementary strand.

In one embodiment, the percent amino acid sequence similarity between an Ftase polypeptide such as SEQ ID NO:2 or SEQ ID NO:177 , and functional equivalents thereof is at least about 60% ($\geq$ 60%). In a preferred embodiment, the percent amino acid sequence similarity between an Ftase polypeptide and its functional equivalents is at least about 75% ($\geq$75%). More preferably, the percent amino acid sequence similarity between an Ftase polypeptide and its functional equivalents is at least about 80%, and still more preferably, at least about 90%, when consecutive amino acids are compared.

Isolated and/or recombinant nucleic acids meeting these criteria comprise nucleic acids having sequences identical to sequences of naturally occurring ERA1 genes and portions thereof, or variants of the naturally occurring genes. Such variants include mutants differing by the addition, deletion or substitution of one or more nucleotides, modified nucleic acids in which one or more nucleotides are modified (e.g., DNA or RNA analogs), and mutants comprising one or more modified nucleotides.

Such nucleic acids, including DNA or RNA, can be detected and isolated by hybridization under high stringency conditions or moderate stringency conditions, for example, which are chosen so as to not permit the hybridization of nucleic acids having non-complementary sequences. "Stringency conditions" for hybridizations is a term of art which refers to the conditions of temperature and buffer concentration which permit hybridization of a particular nucleic acid to another nucleic acid in which the first nucleic acid may be perfectly complementary to the second, or the first and second may share some degree of complementarity which is less than perfect. For example, certain high stringency conditions can be used which distinguish perfectly complementary nucleic acids from those of less complementarity. "High stringency conditions" and "moderate stringency conditions" for nucleic acid hybridizations are explained on pages 2.10.1-2.10.16 (see particularly 2.10.8-11) and pages 6.3.1-6 in *Current Protocols in Molecular Biology* (Ausubel, F.M. *et al.*, eds., Vol. 1, containing supplements up through Supplement 29, 1995), the teachings of which are hereby incorporated by reference. The exact conditions which determine the stringency of hybridization depend not only on ionic strength, temperature and the concentration of

destabilizing agents such as formamide, but also on factors such as the length of the nucleic acid sequence, base composition, percent mismatch between hybridizing sequences and the frequency of occurrence of subsets of that sequence within other non-identical sequences. Thus, high or moderate stringency conditions can be determined empirically.

High stringency hybridization procedures can (1) employ low ionic strength and high temperature for washing, such as 0.015 M NaCl/ 0.0015 M sodium citrate, pH 7.0 (0.1X SSC) with 0.1% sodium dodecyl sulfate (SDS) at 50°C; (2) employ during hybridization 50% (vol/vol) formamide with 5X Denhardt's solution (0.1% weight/volume highly purified bovine serum albumin/ 0.1% wt/vol Ficoll/ 0.1% wt/vol polyvinylpyrrolidone), 50 mM sodium phosphate buffer at pH 6.5 and 5X SSC at 42°C; or (3) employ hybridization with 50% formamide, 5X SSC, 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5X Denhardt's solution, sonicated salmon sperm DNA (50 μg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2X SSC and 0.1% SDS. Moderate stringency conditions would be similar except that hybridization would employ 25% formamide in place of 50% formamide.

By varying hybridization conditions from a level of stringency at which no hybridization occurs to a level at which hybridization is first observed, conditions which will allow a given sequence to hybridize with the most similar sequences in the sample can be determined.

Exemplary conditions are described in Krause, M.H. and S.A. Aaronson (1991) *Methods in Enzymology, 200*:546-556. Also, see especially page 2.10.11 in *Current Protocols in Molecular Biology* (*supra*), which describes how to determine washing conditions for moderate or low stringency conditions. Washing is the step in which conditions are usually set so as to determine a minimum level of complementarity of the hybrids. Generally, from the lowest temperature at which only homologous hybridization occurs, a 1% mismatch between hybridizing nucleic acids results in a 1°C decrease in the melting temperature T_m, for any chosen SSC concentration. Generally, doubling the concentration of SSC results in an increase in T_m of ≈17°C. Using these guidelines, the washing temperature can be determined empirically for moderate or low stringency, depending on the level of mismatch sought.

Isolated and/or recombinant nucleic acids that are characterized by their ability to hybridize to (a) a nucleic acid encoding an Ftase polypeptide, such as the nucleic acids depicted as SEQ ID NO:1 or 172, (b) the complement of SEQ ID NO:1 or 172, (c) or a portion of (a) or (b) (*e.g.* under high or moderate stringency conditions), may further encode a protein or polypeptide having at least one functional characteristic of an Ftase polypeptide, such as

regulation of lateral branching under diurnal light cycles, or regulation of the response to ABA, or regulation of senescence.

Enzymatic assays, complementation tests, or other suitable methods can also be used in procedures for the identification and/or isolation of nucleic acids which encode a polypeptide such as a polypeptide of the amino acid sequence SEQ ID NO:2 or SEQ ID NO:177 or a functional equivalent or fragment thereof of this polypeptide. The antigenic properties of proteins or polypeptides encoded by hybridizing nucleic acids can be determined by immunological methods employing antibodies that bind to an Ftase polypeptide such as immunoblot, immunoprecipitation and radioimmunoassay. PCR methodology, including RAGE (Rapid Amplification of Genomic DNA Ends), can also be used to screen for and detect the presence of nucleic acids which encode Ftase-like proteins and polypeptides, and to assist in cloning such nucleic acids from genomic DNA. PCR methods for these purposes can be found in Innis, M.A., *et al.* (1990) *PCR Protocols: A Guide to Methods and Applications*, Academic Press, Inc., San Diego, CA., incorporated herein by reference.

The nucleic acids described herein are used in the methods of the present invention for production of proteins or polypeptides which are incorporated into cells, tissues, plant parts, plants and other photosynthetic organisms. In one embodiment, DNA containing all or part of the coding sequence for an Ftase polypeptide, or DNA which hybridizes to DNA having the sequence SEQ ID NO:2 or SEQ ID NO:177 is incorporated into a vector for expression of the encoded polypeptide in suitable host cells. The encoded polypeptide consisting of an Ftase subunit or its functional equivalent is capable of farnesyl transferase activity. The term "vector" as used herein refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked.

Primers and probes consisting of 20 or more contiguous nucleotides of the above-described nucleic acids are also included as part of this invention. Thus, one nucleic acid of this invention comprises a specific sequence of about 20 to about 200 or more nucleotides which are identical or complementary to a specific sequence of nucleotides of the Ftase protein-encoding DNA or transcribed mRNA. These probes and primers can be used to identify and isolate Ftase-encoding nucleic acid from other photosynthetic organisms.

Nucleic acids referred to herein as "isolated" are nucleic acids separated away from the nucleic acids of the genomic DNA or cellular RNA of their source of origin (e.g., as it exists in cells or in a mixture of nucleic acids such as a library), and may have undergone further processing. "Isolated" nucleic acids include nucleic acids obtained by methods described herein, similar methods or other suitable methods, including essentially pure nucleic acids, nucleic acids

produced by chemical synthesis, by combinations of biological and chemical methods, and recombinant nucleic acids which are isolated. Nucleic acids referred to herein as "recombinant" are nucleic acids which have been produced by recombinant DNA methodology, including those nucleic acids that are generated by procedures which rely upon a method of artificial recombination, such as the polymerase chain reaction (PCR) and/or cloning into a vector using restriction enzymes. "Recombinant" nucleic acids are also those that result from recombination events that occur through the natural mechanisms of cells, but are selected for after the introduction to the cells of nucleic acids designed to allow or make probable a desired recombination event. Portions of the isolated nucleic acids which code for polypeptides having a certain function can be identified and isolated by, for example, the method of Jasin, M., *et al.*, U.S. Patent No. 4,952,501.

A further embodiment of the invention is antisense nucleic acids or oligonucleotides which are complementary, in whole or in part, to a target molecule comprising a sense strand, and can hybridize with the target molecule. The target can be DNA, or its RNA counterpart (*i.e.*, wherein T residues of the DNA are U residues in the RNA counterpart). When introduced into a cell, antisense nucleic acids or oligonucleotides can inhibit the expression of the gene encoded by the sense strand or the mRNA transcribed from the sense strand. Antisense nucleic acids can be produced by standard techniques. See, for example, Shewmaker, *et al.*, U.S. Patent No. 5,107,065.

In a particular embodiment, an antisense nucleic acid or oligonucleotide is wholly or partially complementary to and can hybridize with a target nucleic acid (either DNA or RNA), wherein the target nucleic acid can hybridize to a nucleic acid having the sequence of the complement of the strand in SEQ ID NO:1 or 172. For example, an antisense nucleic acid or oligonucleotide can be complementary to a target nucleic acid having the sequence shown as the strand of the open reading frame of SEQ ID NO:1 or 172, or nucleic acid encoding a functional equivalent or fragment thereof of Ftase, or to a portion of these nucleic acids sufficient to allow hybridization. A portion, for example, a sequence of 16 nucleotides could be sufficient to inhibit expression of the protein. Fragments comprising 25 or more consecutive nucleotides complementary to SEQ ID NO:1 or 172 could also be used. Or, an antisense nucleic acid or oligonucleotide complementary to 5' or 3' untranslated regions, or overlapping the translation initiation codon (5' untranslated and translated regions), of the ERA1 gene, or a gene encoding a functional equivalent or fragment thereof can also be effective. In another embodiment, the antisense nucleic acid is wholly or partially complementary to and can hybridize with a target nucleic acid which encodes an Ftase polypeptide.

In addition to the antisense nucleic acids of the invention, oligonucleotides can be constructed which will bind to duplex nucleic acid either in the gene or the DNA:RNA complex of transcription, to form a stable triple helix-containing or triplex nucleic acid to inhibit transcription and/or expression of a gene encoding an Ftase polypeptide or its functional equivalent. Frank-Kamenetskii, M.D. and Mirkin, S.M. (1995) *Ann. Rev. Biochem. 64*:65-95. Such oligonucleotides of the invention are constructed using the base-pairing rules of triple helix formation and the nucleotide sequence of the gene or mRNA for Ftase. These oligonucleotides can block Ftase- type activity in a number of ways, including prevention of transcription of the ERA1 gene or by binding to mRNA as it is transcribed by the gene.

Another aspect of the invention pertains to the use of post transcriptional gene silencing (PTGS) to repress gene expression. Double stranded RNA can initiate the sequence specific repression of gene expression in plants and animals. Double stranded RNA is processed to short duplex oligomers of 21-23 nucleotides in length. These small interfering RNA's suppress the expression of endogenous and heterologous genes in a sequence specific manner (Fire et al. Nature 391:806-811, Carthew, Curr. Opin. in Cell Biol., 13:244-248, Elbashir et al., Nature 411:494-498). A RNAi suppressing construct can be designed in a number of ways, for example, transcription of a inverted repeat which can form a long hair pin molecule, inverted repeats separated by a spacer sequence that could be an unrelated sequence such as GUS or an intron sequence. Transcription of sense and antisense strands by opposing promoters or cotranscription of sense and antisense genes.

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Another aspect of the invention pertains to the use of the dominant-negative genetic approach. Briefly the presence of a dominant trait, i.e. the expression of a transgene, results in a

reduction of enzyme activity or reduced production of the enzymatic end-product. It has been demonstrated that FT is a heterodimer formed by α- and β- subunits. FT activity relies on the proper dimerization between these subunits to form functional enzyme. Expression of a non-functional subunit will interact with the second subunit to produce a non-functional enzyme and hence reduced enzymatic activity. The non-functional aspect may be in respect to, but not limited to, subunit interaction, substrate binding or enzyme catalysis, for example. Alternatively the expressed trait may produce a substrate analogue which competes with native substrate, the end result being decreased farnesylation of biologically active substrate.

The invention also relates to proteins or polypeptides encoded by the novel nucleic acids described herein. The proteins and polypeptides of this invention can be isolated and/or recombinant. Proteins or polypeptides referred to herein as "isolated" are proteins or polypeptides purified to a state beyond that in which they exist in cells. In a preferred embodiment, they are at least 10% pure; *i.e.*, substantially purified. "Isolated" proteins or polypeptides include proteins or polypeptides obtained by methods described *infra*, similar methods or other suitable methods, and include essentially pure proteins or polypeptides, proteins or polypeptides produced by chemical synthesis or by combinations of biological and chemical methods, and recombinant proteins or polypeptides which are isolated. Proteins or polypeptides referred to herein as "recombinant" are proteins or polypeptides produced by the expression of recombinant nucleic acids.

In a preferred embodiment, the protein or portion thereof has at least one function characteristic of an Ftase; for example, catalytic activity affecting, *e.g.*, normal lateral branching, florets/inflorescence, seed germination, or stomatal opening, and binding function, and/or antigenic function (*e.g.*, binding of antibodies that also bind to naturally occurring Ftase). As such, these proteins are referred to as Ftases of plant origin, and include, for example, naturally occurring Ftase, variants (*e.g.* mutants) of those proteins and/or portions thereof. Such variants include mutants differing by the addition, deletion or substitution of one or more amino acid residues, or modified polypeptides in which one or more residues are modified, and mutants comprising one or more modified residues.

The invention also relates to isolated and/or recombinant portions of an Ftase as described above, especially the β subunit of an Ftase protein. Portions of the enzyme can be made which have full or partial function on their own, or which when mixed together (though fully, partially, or nonfunctional alone), spontaneously assemble with one or more other

polypeptides to reconstitute a functional protein having at least one functional characteristic of an Ftase of this invention.

A number of genes have been identified that are induced by ABA. This suggests that ABA-induced tolerance to adverse environmental conditions is a complex multigenic event. Thus, identification and transfer of single genes into crop plants which improves the viability of the plant under different environmental conditions due to increased responsiveness to ABA is novel and extremely useful.

To identify genes that could be more global controllers of ABA-regulated plant processes, genetic screens were applied in a number of plant species to isolate mutations that alter the response of the plant to the hormone.

Mutations that confer enhanced response to ABA (*era*) in *Arabidopsis* seeds were identified by their ability to prevent seed germination with low concentrations of ABA that normally permit wild-type (controls, *i.e.*, naturally-occurring) seed germination. Of these, the *era*1 mutant class, which includes one transferred DNA (T-DNA) line (*era*1-1, ecotype Wassilewskija) and two neutron-generated mutants (*era*1-2 and *era*1-3, ecotype Columbia), was of added interest because this class showed decreased germination efficiency under normal postimbibition. Mutations that enhance ABA responsiveness should, in principle, be more dormant. Dormancy in *era*1 alleles was alleviated by a 4-day chilling period; the efficiency of *era*1 germination increased with the length of time the seeds are chilled. In many plant species, breaking dormancy to allow germination requires vernalization and exposure to moist, low-temperature environments for an extended period (Baskin and Baskin, 1971). The germination profile of *era* mutants could reflect an increased state of ABA-induced dormancy; consequently, these seeds require longer vernalization to germinate. Support for this contention came from construction of double mutants of *era1* with both ABA biosynthetic (*aba1-1*) and insensitive mutants (*abi1-1* and *abi3-6*). In all cases, the double mutants had reduced dormancy as compared with *era* 1, indicating that the increased dormancy observed in *era*1 seed was dependent on ABA synthesis or sensitivity.

Aside from broadening the spectrum of new ABA response mutants, supersensitivity screens were also used to identify negative regulators of ABA sensitivity. That is, inhibition of these gene functions enhances the ABA response. One of these genes (ERA1) has been cloned and demonstrated to encode the β-subunit of a heterodimeric protein farnesyl transferase (Ftase) (Cutler *et al* ., 1996). The *era*1-1 mutation, which is due to a T-DNA insertion, allowed the isolation of plant genomic regions flanking the insertions. Using the flanking regions as probes, the wild-type cDNA and genomic clones were isolated. Sequence analysis of these described a

gene encompassing 3.5 kb of genomic DNA. The gene contains 13 introns which are underlined in Figures 1A-1C and the T-DNA insertion site in *era1-1* is in intron 8. Southern (DNA) analysis of wild-type DNA, *era1-2*, and *era1-3* probed with *Era1*cDNA revealed that both fast-neutron alleles contain deletions spanning the *ERA1* locus. Fast-neutron mutagenesis induced small deletions in *Arabidopsis* (Shirley *et al.*, 1992), and subsequent genomic analysis with a 14-kb probe that spans the *ERA*1 locus determined the size of the *era*1-2 deletion to be about 7.5 kb and the *era*1-3 deletion to be slightly larger. Thus all three *era*1 alleles contained DNA disruptions at the same locus, confirming the identity of the ERA locus.

Conceptual translation of the longest open reading frame (404 amino acids) in the ERA1 gene produced a protein (Figures 2 and 4) with a high sequence similarity to yeast, pea, and mammalian protein farnesyl transferase β subunit genes (Goodman *et al.*, 1988; Chen *et al.*, 1991; Yang *et al.*, 1993). Farnesyl transferases consist of α and β subunits that dimerize, forming an enzyme that catalyzes the attachment of farnesyl pyrophosphate (15 carbons) to proteins containing a COOH-terminal CaaX motif (Schafer and Rine, 1992), where C designates cysteine residue, aa is usually aliphatic amino acids, and X may designate a cysteine, serine, methionine, or glutamine residue. Both plant β subunit genes contain a region of about 50 amino acids near their COOH-terminus that is absent in yeast and animal β subunit genes.

In yeast and mammalian systems, Ftases modify several signal transduction proteins for membrane localization. This is achieved by the attachment of the lipophilic farnesyl sidechain to the protein target via the Ftase. The attachment of the farnesyl group causes a change in the overall hydrophobicity of the target allowing the protein to anchor itself into the membrane where it usually interacts with other signal transduction molecules. That the loss of farnesylation activity in the *era*1 mutant leads to an enhanced response of the seed to ABA suggests a target protein in *Arabidopsis* must be localized to the membrane to attenuate the ABA signal. Thus farnesylation in *Arabidopsis*, appears to be required for the normal function of a negative regulator of ABA sensitivity.

Subsequent work has shown that loss of ERA1 gene function in *Arabidopsis* confers an enhanced tolerance to environmental stresses at the level of the mature plant. For example, a comparison of wild-type plants and *era*1 mutant plants grown in soil under standard laboratory conditions (24 hr light, 150 μE $m^{-2}sec^{-1}$, 30% humidity) showed that the mutants did not require water as frequently as the wild-type plants in order to maintain viability (Figure 5). When mutant and wild-type plants were grown until flowering occurred, watering was stopped

and the plants were observed each subsequent day for signs of stress. Water loss was significantly reduced in the mutant plants compared to the wild-type plants (Figures 6 and 7).

To determine if the observed increased drought tolerance of *era* mutants was related to ERA1 gene function, transgenic plants containing a ERA1 promoter fusion to a reporter GUS gene (made by inserting a 5 Kb fragment of the ERA1 promoter into a promoterless GUS T-DNA plasmid), were constructed. Analysis of the transgenic plants showed that ERA1 is transcriptionally expressed in the epidermal tissue of *Arabidopsis* and that this expression is guard-cell specific. Expression of ERA1 was also noted in the meristematic tissue of the plants and in root hairs. The guard cell expression of ERA1 is consistent with the drought tolerance of the mutant as these cells are the major regulators of water transpiration through the plant. It would be expected that ERA1-regulated stomatal conductance would require expression of the ERA1 gene in the guard cells. Hence loss of ERA1 gene function results in guard cells which are more responsive to ABA which, in turn, leads to more drought responsive guard cell regulation. Therefore, modification of Ftase expression or activity in higher plants, especially crop plants, will have profound effects on stomatal conductance and transpiration rates in the plants.

The nature of the *era*1 mutation in *Arabidopsis* demonstrates that inhibition of farnesylation will enhance ABA responses in a plant and alteration of this enzyme activity in crop species. Inhibition of Ftase activity in crop plants can be achieved via a number of methods. For example, antisense technology of cognate ERA1 genes in a variety of crop species can be used to reduce Ftase activity, thus increasing drought tolerance. By specifically producing ERA1 antisense RNA in guard cells, the amount of Ftase synthesized can be reduced to a level which would mimic era mutant phenotypes. The ERA1 promoter is regulated in a number of different tissues ranging from shoot meristems to root hairs. By determining the elements of the ERA1 promoter which allow expression in specific tissues, it is possible to tailor the expression of antisense ERA1 to only one tissue or cell type, such as guard cells.

Another method to inhibit Ftase activity in plants is the production of specific peptide inhibitors of farnesylation in transgenic plants. In mammalian and yeast systems, the carboxyl terminal target sequence (CaaX, where C=cysteine, x=aliphatic, X=any amino acid) which allows the attachment of the farnesyl group to specific proteins has been clearly defined. Peptides which mimic these target sequences have been made and shown to inhibit farnesylation of the endogenous target proteins in these systems. Moreover, CAIM is farnesylated *in vivo* in *Arabidopsis*. Thus, similar inhibitors can be applied to higher plants to competitively inhibit Ftase *in vivo*. Again, this can be done through expression of inhibitor peptides in transgenic

plants by synthesizing the DNA sequence for a CaaX peptide and fusing it to a guard cell-specific promoter. In both methods, using the appropriate promoters, antisense Ftase or peptide inhibitors can be specifically targeted and controlled.

Also included in the invention are methods of producing a transgenic plant. The method includes introducing into one or more plant cells a compound that alters, e.g., inhbits farnesylation of a polypeptide having a carboxyl terminal CaaX motif in the plant to generate a transgenic plant cell and regenerating a transgenic plant from the transgenic cell. In some aspects the compound alters, e.g., increases or decreases CaaX prenyl protease expression or activity. Alternatively, the compound alters farnesyltransferase expression or activity. In other aspects the compound alters isoprenylcysteine carboxyl methytransferase expression or activity. The compound can be, *e.g.*, (i) a CaaX prenyl protease, farnesyltransferase or isoprenylcysteine carboxyl methytransferase polypeptide; (ii) a nucleic acid encoding a CaaX prenyl protease, farnesyltransferase or isoprenylcysteine carboxyl methytransferase polypeptide; (iii) a nucleic acid that increases expression of a nucleic acid that encodes a CaaX prenyl protease, farnesyltransferase or isoprenylcysteine carboxyl methytransferase polypeptide ; (iv) a nucleic acid that decreases the expression of a nucleic acid that encodes a CaaX prenyl protease, farnesyltransferase or isoprenylcysteine carboxyl methytransferase polypeptide; (v) a CaaX prenyl protease, farnesyltransferase or isoprenylcysteine carboxyl methytransferase antisense nucleic acid and derivatives, fragments, analogs and homologs thereof. A nucleic acid that increases expression of a nucleic acid that encodes a CaaX prenyl protease, farnesyltransferase or isoprenylcysteine carboxyl methytransferase polypeptide includes, *e.g.*, promoters, enhancers. The nucleic acid can be either endogenous or exogenous. Preferably, the compound is a CaaX prenyl protease, farnesyltransferase or isoprenylcysteine carboxyl methytransferase polypeptide or a nucleic acid encoding a CaaX prenyl protease, farnesyltransferase or isoprenylcysteine carboxyl methytransferase polypeptide.

Included in the invention are methods of producing a transgenic plant that has increased stress resistance, delayed senesense or increased sensitivity to ABA. The method includes introducing into one or more plant cells a compound that alters farnesyl transferase expression (i.e. farnesyl transferase alpha or beta) or activity in the plant. The compound can be, *e.g.*, (i) a farnesyl transferase polypeptide inhibitor; (ii) a nucleic acid encoding a farnesyl transferase polypeptide inhibitor; (iii) a nucleic acid that decreases expression of a nucleic acid that encodes a farnesyl transferase polypeptide and, derivatives, fragments, analogs and homologs thereof; (iv) an antisense farnesyl transferase nucleic acid. A nucleic acid that decreases expression of a nucleic acid that encodes a farnesyl transferase polypeptide includes, *e.g.*, antisense nucleic

acids or RNA inhibitory nucleic acids. The nucleic acid can be either endogenous or exogenous. Preferably the compound is a farnesyl transferase polypeptide or a nucleic acid encoding a farnesyl transferase polypeptide. More preferably the compound is a nucleic acid complementary to a nucleic acid encoding a farnesyl transferase polypeptide. For example an anti-sense nucleic acid molecule.

Alternatively the compound is a nucleic acid molecule comprising a nucleic acid sequence enocoding a mutated farnesyl transferase, isoprenylcysteine carboxyl methytransferase or CaaX prenyl protease polypeptide. By mutated is meant that the polypeptide lacks one or more function of a wild-type polypeptide. For example, a mutated farnesyltransferase beta polypeptide is a polypeptide has less amino acids than a full length wild type polypeptide by still retains the ability to dimerize with an alpha subunit. For example a mutated farnesytransferase beta polypeptide is less than 314 amino acids in length. Preferably, the mutated farnesytransferase beta polypeptide comprises the amino acid sequence of SEQ ID NO:1 or a fragement thereof.

In another aspect the compound is a nucleic acid encoding a CaaX motif. Alternatively, the CaaX motif is operably liked to a promoter.

Also included in the invention is a plant where a mutation has been introduced in the gene encoding farnesyl transferase (i.e. alpha or beta) which results in a plant that has decreased farnesyl transferase acitivity and increased tolerase to stress as compared to a wild type plant. The mutation may be introduced by chemical or mechanical means.

In various aspects the transgenic plant has an altered phenotype as compared to a wild type plant (*i.e.*, untransformed). By altered phenotype is meant that the plant has a one or more characteristic that is different from the wild type plant. For example, the transgenic plant has an increased resistence to stress. Increased stress resistance is meant that the transgenic plant can grow under stress conditions (*e.g.,* high salt, decreased water, low temperatures, high temperatures) or under conditions that normally inhibit the growth of an untransformed Stresses include, for example, chilling stress, heat stress, heat shock, salt stress, water stress (*i.e,* drought), nutritional stress, disease, grazing pests, wound healing, pathogens such as for example fungi, bacteria, nematodes, viruses or parasitic weed and herbicides. Methodologies to determine plant growth or response to stress include for example, height measurements, weight or biomass measurements, leaf area or number, ability to flower, water use, transpiration rates and yield. Alternatively, the transformed plant has an increased (*i.e.,* enhanced) ABA sensitivity. The enhanced ABA sensitivity is at the seedling growth stage. Alternatively, the

enhanced ABA sensitivity is at the mature plant stage. Additional altered phenotypes include for example, enhanced vegetative growth (*e.g.*, increased leaf number, thickness and overall biomass), delayed reproductive growth (*e.g.*, flowering later); enhanced seedling vigor (*e.g.*,increased root biomass and length), enhanced lateral root formation and therefore soil penetration more extensive vascular system resulting in an enhanced transport system.

The plant can be any plant type including, for example, species from the genera *Cucurbita, Rosa, Vitis, Juglans, Fragaria, Lotus, Medicago, Onobrychis, Trifolium, Trigonella, Vigna, Citrus, Linum, Geranium, Manihot, Daucus, Arabidopsis, Brassica, Raphanus, Sinapis, Atropa, Capsicum, Datura, Hyoscyamus, Lycopersicon, Nicotiana, Solanum, Petunia, Digitalis, Majorana, Ciahorium, Helianthus, Lactuca, Bromus, Asparagus, Antirrhinum, Heterocallis, Nemesis, Pelargonium, Panieum, Pennisetum, Ranunculus, Senecio, Salpiglossis, Cucumis, Browaalia, Glycine, Pisum, Phaseolus, Lolium, Oryza, Zea, Avena, Hordeum, Secale, Triticum, Sorghum, Gossypium, Picea, Caco,* and *Populus.*

This invention provides a method of producing drought-tolerant plants comprising: preparing a nucleic acid construct which comprises a promoter operably-linked to a nucleic acid comprising or encoding antisense to SEQ ID NO: 1, 14, 40, 43, 80-85 or 172, or nucleic acid comprising a functional equivalent or fragment thereof of the antisense; inserting the nucleic acid construct into a vector; transforming a plant, tissue culture, or plant cells with the vector; and growing the plant or regenerating a plant from the tissue culture or plant cells; wherein drought-tolerant plants are produced. This method can be used wherein the nucleic acid is selected from the group consisting of 25-200 or more consecutive nucleotides complementary to SEQ ID NO: 1, 14, 40, 43, 80-85 or 172, oligonucleotides consisting of 25 or more consecutive nucleotides of SEQ ID NO: 1, 14, 40, 43, 80-85 or 172 or its complement, or nucleic acid encoding a peptide inhibitor of farnesyl transferase

In addition to stomatal regulation which is extremely sensitive to ABA, *era* plants also demonstrate delayed senescence under drought conditions, indicating that farnesylation negatively regulates a number of drought-induced responses in *Arabidopsis*. The *era* plants grown under normal laboratory conditions take longer to turn yellow. The mutant plants remained green and viable long after the wild-type had senesced and died. Detached leaves of an *era* mutant plant do not yellow as quickly as detached leaves of wild-type plants (Figure 8). Similar-sized leaves which were developmentally identical were taken from wild-type and *era* plants and placed on agar-containing petri plates (See Example 7). Normally, a wild-type leaf begins to lose chlorophyll about five days later and eventually bleachs. The leaves of the mutant

plants remained green for twice as long. Because the leaves were in constant contact with the agar they were not drought stressed, indicating the reduced senescence of the *era1* mutant is not a drought-induced phenomenon.

Moreover, under a 10 hr day/16 hr night cycle, the plant life cycle can be doubled versus the wild-type plants (3 months). It appears therefore, that chlorophyll turnover and senescence signals are altered in the *era*1 mutant. For example, wild-type and mutant plants were grown in pots under well-watered conditions to stages of development where the wild-type plant leaves would begin to senesce (about the time of flower development). At this time, developmentally-similar leaves were assayed for senescence-induced marker genes by northern blot analysis (Example 8). Two genes, SAG12 and SAG13, in which transcription is normally induced during senescence in wild-type plants, were not induced in the *era1* mutant (Figure 9). Further, CAB transcription is maintained (Figure 9). Taken together, these results indicate the senescence induction program in *era1* mutants is delayed compared to wild-type plants, showing that loss of farnesylation activity causes a retardation of the induction of senescence in the plant even under conditions wherein water stress is not an environmental factor.

In addition to effects on senescence and water loss, the *era*1 mutants show a difference in branching and flowering habit when grown under diurnal light cycles. Under continuous (24 hours light/day) light, the branching pattern of mutants does not differ from that of wild-type plants. However, when given a dark period, the mutants do not produce as many lateral branches as wild-type plants. When measured, plants with loss of farnesylation activity produced only 2.4 branches per plant compared to 3.6 lateral branches per wild-type plant. This represents a 30% decrease in lateral branches per plant.

Flowering is affected by loss of Ftase activity as well. Plants lacking Ftase activity produce more flowers per plant (25-30 buds/inflorescence) than wild-type plants (10-15 buds/inflorescence). Thus, on average there are twice as many flower buds are present on the mutants than on the wild-type plants.

These pleiotrophic effects of the *era1* loss of function mutants on whole plant development indicate that the ERA1 gene can be a coordinate regulator of a collection of plant developmental functions.

Until now, there was no known function for farnesylation in higher plants, including a role in ABA signal transduction. Ftases have been found in a number of higher plants such as tomato and pea, so it is clear that this enzyme has functions across species boundaries. Furthermore, overproduction of farnesyl transferase target peptides or the use of farnesylation inhibitors completely inactivates Ftase in mammalian and yeast systems. Thus, similar

inhibitors can be applied to higher plants to inactivate Ftase *in vivo*. In both cases with the appropriate promoters, antisense Ftase or peptide inhibitors can be specifically targeted and controlled.

The farnesylation deficient mutants are also supersensitive to exogenous auxin. That these mutants show reduced branching and minor alterations in meristem organization, can be explained by altered auxin regulation. Thus other hormone functions are affected in this mutant, which indicates that, in addition to ABA pathways, other hormone regulated pathways are controlled by Ftase activity. These results demonstrate that the ERA1 gene provides a molecular mechanism to coordinate regulation of different hormone signaling molecules.

In accordance with the present invention, the plants included within the scope of this invention are higher and lower plants of the plant kingdom. Mature plants, seedlings and seeds are included in the scope of the invention. A mature plant includes a plant at any stage in development beyond the seedling. A seedling is a very young, immature plant in the early stages of development. Plant parts, protoplasts and tissue culture are also provided by this invention.

Transgenic plants are included within the scope of the present invention which have the phenotype characterized by the *era*1 mutation. Seed of transgenic plants are provided by this invention and can be used to propagate more plants containing the constructs of this invention.

ERA1 function in a number of crop plants can be inhibited to enhance the plant's response to adverse environmental conditions that require ABA-mediated signaling. Control of farnesylation in higher plants regulates both embryonic and vegetative tissue response to this hormone (Cutler, *et al.*, 1996). The increased sensitivity translates into a faster response of the tissue to stress conditions which in turn confers increased protection of the plant to the environmental stress. Because this only requires the control of a single gene, ERA1, it should be possible to control farnesylation in a variety of plants by controlling the synthesis or activity of this enzyme. Furthermore, the work described herein clearly indicates that altering the ABA signal transduction pathway by manipulating the genes that control the ABA response makes it possible to improve the plant's response to adverse water stress conditions.

To produce transgenic plants of this invention, a construct comprising the gene encoding Ftase, or nucleic acid encoding its functional equivalent, and a promoter are incorporated into a vector through methods known and used by those of skill in the art. The promoter can comprise all or part of SEQ ID NO:3. The construct can also include any other necessary regulators such as terminators or the like, operably linked to the coding sequence. It can also be beneficial to

include a 5' leader sequence, such as the untranslated leader from the coat protein mRNA of alfalfa mosaic virus (Jobling, S.A. and Gehrke, L. (1987) *Nature 325*:622-625) or the maize chlorotic mottle virus (MCMV) leader (Lommel, S.A., *et al.* (1991) *Virology 81*:382-385). Those of skill in the art will recognize the applicability of other leader sequences for various purposes. Exemplary constructs include SEQ ID NO: 54 -64.

Targeting sequences are also useful and can be incorporated into the constructs of this invention. A targeting sequence is usually translated into a peptide which directs the polypeptide product of the coding nucleic acid sequence to a desired location within the cell, such as to the plastid, and becomes separated from the peptide after transit of the peptide is complete or concurrently with transit. Examples of targeting sequences useful in this invention include, but are not limited to, the yeast mitochondrial presequence (Schmitz, *et al.* (1989) *Plant Cell 1*:783-791), the targeting sequence from the pathogenesis-related gene (PR-1) of tobacco (Cornellisen, *et al.* (1986) *EMBO J. 5*:37-40), vacuole targeting signals (Chrispeels, M.J. and Raikhel, N.V. (1992) *Cell 68*:613-616), secretory pathway sequences such as those of the ER or Golgi (Chrispeels, M.J. (1991) *Ann. Rev. Plant Physiol. Plant Mol. Biol. 42*:21-53). Intraorganellar sequences may also be useful for internal sites, *e.g.*, thylakoids in chloroplasts. Theg, S.M. and Scott, S.V. (1993) *Trends in Cell Biol. 3*:186-190.

In addition to 5' leader sequences, terminator sequences are usually incorporated into the construct. In plant constructs, a 3' untranslated region (3' UTR) is generally part of the expression plasmid and contains a polyA termination sequence. The termination region which is employed will generally be one of convenience, since termination regions appear to be relatively interchangeable. The octopine synthase and nopaline synthase termination regions, derived from the Ti-plasmid of *A. tumefaciens*, are suitable for such use in the constructs of this invention.

Any suitable technique can be used to introduce the nucleic acids and constructs of this invention to produce transgenic plants with an altered genome. For grasses such as maize, microprojectile bombardment (see for example, Sanford, J.C., *et al.*, U.S. Patent No. 5,100,792 (1992) can be used. In this embodiment, a nucleotide construct or a vector containing the construct is coated onto small particles which are then introduced into the targeted tissue (cells) via high velocity ballistic penetration. The vector can be any vector which permits the expression of the exogenous DNA in plant cells into which the vector is introduced. The transformed cells are then cultivated under conditions appropriate for the regeneration of plants, resulting in production of transgenic plants.

Transgenic plants carrying the construct are examined for the desired phenotype using a variety of methods including but not limited to an appropriate phenotypic marker, such as antibiotic resistance or herbicide resistance, or visual observation of the time of floral induction compared to naturally-occurring plants.

Other known methods of inserting nucleic acid constructs into plants include Agrobacterium-mediated transformation (see for example Smith, R.H., *et al.*, U.S. Patent No. 5,164,310 (1992)), electroporation (see for example, Calvin, N., U.S. Patent No. 5,098,843 (1992)), introduction using laser beams (see for example, Kasuya, T., *et al.*, U.S. Patent No. 5,013,660 (1991)) or introduction using agents such as polyethylene glycol (see for example Golds, T. *et al.* (1993) *Biotechnology*, 11:95-97), and the like. In general, plant cells may be transformed with a variety of vectors, such as viral, episomal vectors, Ti plasmid vectors and the like, in accordance with well known procedures. The method of introduction of the nucleic acid into the plant cell is not critical to this invention.

The methods of this invention can be used with *in planta* or seed transformation techniques which do not require culture or regeneration. Examples of these techniques are described in Bechtold, N., *et al.* (1993) *CR Acad. Sci. Paris/Life Sciences 316*:118-93; Chang, S.S., *et al.* (1990) *Abstracts of the Fourth International Conference on Arabidopsis Research*, Vienna, p. 28; Feldmann, K.A. and Marks, D.M (1987) *Mol. Gen. Genet. 208*:1-9; Ledoux, L., *et al.* (1985) *Arabidopsis Inf. Serv. 22*:1-11; Feldmann, K.A. (1992) *In*: Methods in *Arabidopsis* Research (Eds. Koncz, C., Chua, N-H, Schell, J.) pp. 274-289; Chee, *et al.*, U.S. patent, Serial No. 5,376,543.

The transcriptional initiation region may provide for constitutive expression or regulated expression. In addition to the ERA1 promoter, many promoters are available which are functional in plants.

Constitutive promoters for plant gene expression include, but are not limited to, the octopine synthase, nopaline synthase, or mannopine synthase promoters from *Agrobacterium*, the cauliflower mosaic virus (35S) promoter, the figwort mosaic virus (FMV) promoter, and the tobacco mosaic virus (TMV) promoter. Constitutive gene expression in plants can also be provided by the glutamine synthase promoter (Edwards, *et al.* (1990) *PNAS 87*:3459-3463), the maize sucrose synthetase 1 promoter (Yang, *et al.* (1990) *PNAS 87*:4144-4148), the promoter from the Rol-C gene of the TLDNA of Ri plasmid (Sagaya, *et al.* (1989) *Plant Cell Physiol. 30*:649-654), and the phloem-specific region of the pRVC-S-3A promoter (Aoyagi, *et al.* (1988) *Mol. Gen. Genet. 213*:179-185).

Heat-shock promoters, the ribulose-1,6-bisphosphate (RUBP) carboxylase small subunit (ssu) promoter, tissue specific promoters, and the like can be used for regulated expression of plant genes. Developmentally-regulated, stress-induced, wound-induced or pathogen-induced promoters are also useful.

The regulatory region may be responsive to a physical stimulus, such as light, as with the RUBP carboxylase ssu promoter, differentiation signals, or metabolites. The time and level of expression of the sense or antisense orientation can have a definite effect on the phenotype produced. Therefore, the promoters chosen, coupled with the orientation of the exogenous DNA, and site of integration of a vector in the genome, will determine the effect of the introduced gene.

Specific examples of regulated promoters also include, but are not limited to, the low temperature Kin1 and cor6.6 promoters (Wang, *et al.* (1995) *Plant Mol. Biol. 28*:605; Wang, *et al.* (1995) *Plant Mol. Biol. 28*:619-634), the ABA inducible promoter (Marcotte Jr., *et al.* (1989) *Plant Cell 1*:969-976), heat shock promoters, such as the inducible hsp70 heat shock promoter of *Drosphilia melanogaster* (Freeling, M., *et al.* (1985) *Ann. Rev. of Genetics 19*: 297-323), the cold inducible promoter from *B. napus* (White, T.C., *et al.* (1994) *Plant Physiol. 106*:917), the alcohol dehydrogenase promoter which is induced by ethanol (Nagao, R.T., *et al.*, Miflin, B.J., Ed. *Oxford Surveys of Plant Molecular and Cell Biology*, Vol. 3, p 384-438, Oxford University Press, Oxford 1986), the phloem-specific sucrose synthase ASUS1 promoter from *Arabidopsis* (Martin, *et al.* (1993) *Plant J. 4*:367-377), the ACS1 promoter (Rodrigues-Pousada, *et al.* (1993) *Plant Cell 5*:897-911), the 22 kDa zein protein promoter from maize (Unger, *et al.* (1993) *Plant Cell 5*:831-841), the ps1 lectin promoter of pea (de Pater, *et al.* (1993) *Plant Cell 5*:877-886), the phas promoter from *Phaseolus vulgaris* (Frisch, *et al.* (1995) *Plant J. 7*:503-512), the lea promoter (Thomas, T.L. (1993) *Plant Cell 5*:1401-1410), the E8 gene promoter from tomato (Cordes, *et al.* (1989) *Plant Cell 1*:1025-1034), the PCNA promoter (Kosugi, *et al.* (1995) *Plant J.* 7:877-886), the NTP303 promoter (Weterings, *et al.* (1995) *Plant J. 8*:55-63), the OSEM promoter (Hattori, *et al.* (1995) *Plant J. 7*:913-925), the ADP GP promoter from potato (Muller-Rober, *et al.* (1994) *Plant Cell 6*:601-604), the Myb promoter from barley (Wissenbach, *et al.* (1993) *Plant J. 4*:411-422), and the plastocyanin promoter from *Arabidopsis* (Vorst, *et al.* (1993) *Plant J. 4*:933-945).

The vector can be introduced into cells by a method appropriate to the type of host cells (*e.g.*, transformation, electroporation, transfection). For the purposes of this disclosure, the terms "transformed with", "transformant", "transformation", "transfect with", and "transfection" all refer to the introduction of a nucleic acid into a cell by one of the numerous methods known

to persons skilled in the art. Transformation of prokaryotic cells, for example, is commonly achieved by treating the cells with calcium chloride so as to render them "competent" to take up exogenous DNA, and then mixing such DNA with the competent cells. Prokaryotic cells can also be infected with a recombinant bacteriophage vector.

Nucleic acids can be introduced into cells of higher organisms by viral infection, bacteria-mediated transfer (*e.g.*, *Agrobacterium* T-DNA delivery system), electroporation, calcium phosphate co-precipitation, microinjection, lipofection, bombardment with nucleic-acid coated particles or other techniques, depending on the particular cell type. For grasses such as corn and sorghum, microprojectile bombardment as described, for example, in Sanford, J.C., *et al.*, U.S. Patent No. 5,100,792 (1992) can be used. Other useful protocols for the transformation of plant cells are provided in Gelvin *et al.*, 1992. Suitable protocols for transforming and transfecting cells are also found in Sambrook *et al.*, 1989. The nucleic acid constructs of this invention can also be incorporated into specific plant parts such as those described *supra* through the transformation and transfection techniques described herein.

To aid in identification of transformed plant cells, the constructs of this invention are further manipulated to include genes coding for plant selectable markers. Useful selectable markers include enzymes which provide for resistance to an antibiotic such as gentamycin, hygromycin, kanamycin, or the like. Similarly, enzymes providing for production of a compound identifiable by color change such as GUS (β- glucuronidase), or by luminescence, such as luciferase, are useful.

For example, antisense Ftase can be produced by integrating a complement of the ERA1 gene linked to DNA comprising SEQ ID NO:3 into the genome of a virus that enters the host cells. By infection of the host cells, the components of a system which permits the transcription of the antisense present in the host cells.

When cells or protoplasts containing the antisense gene driven by a promoter of the present invention are obtained, the cells or protoplasts are regenerated into whole plants. The transformed cells are then cultivated under conditions appropriate for the regeneration of plants, resulting in production of transgenic plants. Choice of methodology for the regeneration step is not critical, with suitable protocols being available for many varieties of plants, tissues and other photosynthetic organisms. See, *e.g.*, Gelvin S.B. and Schilperoort R.A., eds. *Plant Molecular Biology Manual, Second Edition*, Suppl. 1 (1995) Kluwer Academic Publishers, Boston MA, U.S.A.

Transgenic plants carrying the construct are examined for the desired phenotype using a variety of methods including but not limited to an appropriate phenotypic marker, such as

antibiotic resistance or herbicide resistance as described *supra*, or visual observation of their growth compared to the growth of the naturally-occurring plants under the same conditions.

As used herein, the term transgenic plants includes plants that contain either DNA or RNA which does not naturally occur in the wild type (native) plant or known variants, or additional or inverted copies of the naturally-occurring DNA and which is introduced as described herein. Transgenic plants include those into which isolated nucleic acids have been introduced and their descendants, produced from seed, vegetative propagation, cell, tissue or protoplast culture, or the like wherein such alteration is maintained.

Such transgenic plants include, in one embodiment, transgenic plants which are angiosperms, both monocotyledons and dicotyledons. Transgenic plants include those into which DNA has been introduced and their progeny, produced from seed, vegetative propagation, cell, tissue or protoplast culture, or the like.

Seed can be obtained from the regenerated plant or from a cross between the regenerated plant and a suitable plant of the same species. Alternatively, the plant can be vegetatively propagated by culturing plant parts under conditions suitable for the regeneration of such plant parts.

In yet another aspect of this invention are provided plant tissue culture and protoplasts which contain DNA comprising antisense or an altered ERA1 nucleic acid operably linked to an ERA1 promoter, which alters the response of the tissue culture or protoplasts to varying environmental conditions.

The methods of this invention can also be used with *in planta* or seed transformation techniques which do not require culture or regeneration. Examples of these techniques are described in Bechtold, N., *et al.* (1993) *CR Acad. Sci. Paris/Life Sciences 316*:118-93; Chang, S.S., *et al.* (1990) *Abstracts of the Fourth International Conference on Arabidopsis Research*, Vienna, p. 28; Feldmann, K.A. and Marks, D.M (1987) *Mol. Gen. Genet. 208*:1-9; Ledoux, L., *et al.* (1985) *Arabidopsis Inf. Serv. 22*:1-11; Feldmann, K.A. (1992) *In*: Methods in *Arabidopsis* Research (Eds. Koncz, C., Chua, N-H, Schell, J.) pp. 274-289; Chee, *et al.*, U.S. patent, Serial No. 5,376,543.

The isolated nucleic acid molecules of the invention can be used to express PPI protein (e.g., via a recombinant expression vector in a host cell), to detect PPI mRNA (e.g., in a biological sample) or a genetic lesion in a PPI gene, and to modulate PPI activity, as described further, below. In addition, the PPI proteins can be used to screen compounds that modulate the PPI protein activity or expression. In addition, the anti-PPI antibodies of the invention can be used to detect and isolate PPI proteins and modulate PPI activity.

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs) that bind to PPI proteins or have a stimulatory or inhibitory effect on, *e.g.*, PPI protein expression or PPI protein activity. The invention also includes compounds identified in the screening assays described herein.

In one embodiment, the invention provides assays for screening candidate or test compounds which bind to a PPI protein or polypeptide or biologically-active portion thereof. The test compounds of the invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds. *See, e.g.,* Lam, 1997. *Anticancer Drug Design* 12: 145. A "small molecule" as used herein, is meant to refer to a composition that has a molecular weight of less than about 5 kD and most preferably less than about 4 kD. Small molecules can be, *e.g.*, nucleic acids, peptides, polypeptides, peptidomimetics, carbohydrates, lipids or other organic or inorganic molecules. Libraries of chemical and/or biological mixtures, such as fungal, bacterial, or algal extracts, are known in the art and can be screened with any of the assays of the invention.

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt, *et al.*, 1993. *Proc. Natl. Acad. Sci. U.S.A.* 90: 6909; Erb, *et al.*, 1994. *Proc. Natl. Acad. Sci. U.S.A.* 91: 11422; Zuckermann, *et al.*, 1994. *J. Med. Chem.* 37: 2678; Cho, *et al.*, 1993. *Science* 261: 1303; Carrell, *et al.*, 1994. *Angew. Chem. Int. Ed. Engl.* 33: 2059; Carell, *et al.*, 1994. *Angew. Chem. Int. Ed. Engl.* 33: 2061; and Gallop, *et al.*, 1994. *J. Med. Chem.* 37: 1233.

Libraries of compounds may be presented in solution (*e.g.*, Houghten, 1992. *Biotechniques* 13: 412-421), or on beads (Lam, 1991. *Nature* 354: 82-84), on chips (Fodor, 1993. *Nature* 364: 555-556), bacteria (Ladner, U.S. Patent No. 5,223,409), spores (Ladner, U.S. Patent 5,233,409), plasmids (Cull, *et al.*, 1992. *Proc. Natl. Acad. Sci. USA* 89: 1865-1869) or on phage (Scott and Smith, 1990. *Science* 249: 386-390; Devlin, 1990. *Science* 249: 404-406; Cwirla, *et al.*, 1990. *Proc. Natl. Acad. Sci. U.S.A.* 87: 6378-6382; Felici, 1991. *J. Mol. Biol.* 222: 301-310; Ladner, U.S. Patent No. 5,233,409.).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a PPI protein, or a biologically-active portion thereof, is contacted with a test compound and the ability of the test compound to bind to a PPI protein determined. The cell, for example, can be of mammalian origin, plant cell or a yeast cell. Determining the ability of the test compound to bind to the PPI protein can be accomplished, for example, by coupling the test compound with a radioisotope or enzymatic label such that binding of the test compound to the PPI protein or biologically-active portion thereof can be determined by detecting the labeled compound in a complex. For example, test compounds can be labeled with ^{125}I, ^{35}S, ^{14}C, or ^{3}H, either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting. Alternatively, test compounds can be enzymatically-labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product. In one embodiment, the assay comprises contacting a cell which expresses a PPI protein, or a biologically-active portion thereof, with a known compound which binds PPI to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a PPI protein, wherein determining the ability of the test compound to interact with a PPI protein comprises determining the ability of the test compound to preferentially bind to PPI protein or a biologically-active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a PPI protein, or a biologically-active portion thereof, with a test compound and determining the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the PPI protein or biologically-active portion thereof. Determining the ability of the test compound to modulate the activity of PPI or a biologically-active portion thereof can be accomplished, for example, by determining the ability of the PPI protein to bind to or interact with a PPI target molecule. As used herein, a "target molecule" is a molecule with which a PPI protein binds or interacts in nature, for example, a molecule on the surface of a cell which expresses a PPI interacting protein, a molecule on the surface of a second cell, a molecule in the extracellular milieu, a molecule associated with the internal surface of a cell membrane or a cytoplasmic molecule. A PPI target molecule can be a non-PPI molecule or a PPI protein or polypeptide of the invention In one embodiment, a PPI target molecule is a component of a signal transduction pathway that facilitates transduction of an extracellular signal (*e.g.* a signal generated by binding of a compound to a membrane-bound molecule) through the cell membrane and into the cell. The target, for example, can be a second intercellular protein that

has catalytic activity or a protein that facilitates the association of downstream signaling molecules with PPI.

Determining the ability of the PPI protein to bind to or interact with a PPI target molecule can be accomplished by one of the methods described above for determining direct binding. In one embodiment, determining the ability of the PPI protein to bind to or interact with a PPI target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (*i.e.* intracellular Ca^{2+}, diacylglycerol, IP_3, etc.), detecting catalytic/enzymatic activity of the target an appropriate substrate, detecting the induction of a reporter gene (comprising a PPI-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a cellular response, for example, cell survival, cellular differentiation, or cell proliferation.

In yet another embodiment, an assay of the invention is a cell-free assay comprising contacting a PPI protein or biologically-active portion thereof with a test compound and determining the ability of the test compound to bind to the PPI protein or biologically-active portion thereof. Binding of the test compound to the PPI protein can be determined either directly or indirectly as described above. In one such embodiment, the assay comprises contacting the PPI protein or biologically-active portion thereof with a known compound which binds PPI to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a PPI protein, wherein determining the ability of the test compound to interact with a PPI protein comprises determining the ability of the test compound to preferentially bind to PPI or biologically-active portion thereof as compared to the known compound.

In still another embodiment, an assay is a cell-free assay comprising contacting PPI protein or biologically-active portion thereof with a test compound and determining the ability of the test compound to modulate (*e.g.* stimulate or inhibit) the activity of the PPI protein or biologically-active portion thereof. Determining the ability of the test compound to modulate the activity of PPI can be accomplished, for example, by determining the ability of the PPI protein to bind to a PPI target molecule by one of the methods described above for determining direct binding. In an alternative embodiment, determining the ability of the test compound to modulate the activity of PPI protein can be accomplished by determining the ability of the PPI protein further modulate a PPI target molecule. For example, the catalytic/enzymatic activity of the target molecule on an appropriate substrate can be determined as described above.

In yet another embodiment, the cell-free assay comprises contacting the PPI protein or biologically-active portion thereof with a known compound which binds PPI protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a PPI protein, wherein determining the ability of the test compound to interact with a PPI protein comprises determining the ability of the PPI protein to preferentially bind to or modulate the activity of a PPI target molecule.

The cell-free assays of the invention are amenable to use of both the soluble form or the membrane-bound form of PPI protein. In the case of cell-free assays comprising the membrane-bound form of PPI protein, it may be desirable to utilize a solubilizing agent such that the membrane-bound form of PPI protein is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton® X-100, Triton® X-114, Thesit®, Isotridecypoly(ethylene glycol ether)$_n$, N-dodecyl--N,N-dimethyl-3-ammonio-1-propane sulfonate, 3-(3-cholamidopropyl) dimethylamminiol-1-propane sulfonate (CHAPS), or 3-(3-cholamidopropyl)dimethylamminiol-2-hydroxy-1-propane sulfonate (CHAPSO).

In more than one embodiment of the above assay methods of the invention, it may be desirable to immobilize either PPI protein or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to PPI protein, or interaction of PPI protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided that adds a domain that allows one or both of the proteins to be bound to a matrix. For example, GST-PPI fusion proteins or GST-target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, that are then combined with the test compound or the test compound and either the non-adsorbed target protein or PPI protein, and the mixture is incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described, *supra*. Alternatively, the complexes can be dissociated from the matrix, and the level of PPI protein binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either the PPI protein or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated PPI protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well-known within the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with PPI protein or target molecules, but which do not interfere with binding of the PPI protein to its target molecule, can be derivatized to the wells of the plate, and unbound target or PPI protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the PPI protein or target molecule, as well as enzyme-linked assays that rely on detecting an enzymatic activity associated with the PPI protein or target molecule.

In another embodiment, modulators of PPI protein expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of PPI mRNA or protein in the cell is determined. The level of expression of PPI mRNA or protein in the presence of the candidate compound is compared to the level of expression of PPI mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of PPI mRNA or protein expression based upon this comparison. For example, when expression of PPI mRNA or protein is greater (*i.e.*, statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of PPI mRNA or protein expression. Alternatively, when expression of PPI mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of PPI mRNA or protein expression. The level of PPI mRNA or protein expression in the cells can be determined by methods described herein for detecting PPI mRNA or protein.

In yet another aspect of the invention, the PPI proteins can be used as "bait proteins" in a two-hybrid assay or three hybrid assay (*see, e.g.*, U.S. Patent No. 5,283,317; Zervos, *et al.*, 1993. *Cell* 72: 223-232; Madura, *et al.*, 1993. *J. Biol. Chem.* 268: 12046-12054; Bartel, *et al.*, 1993. *Biotechniques* 14: 920-924; Iwabuchi, *et al.*, 1993. *Oncogene* 8: 1693-1696; and Brent WO 94/10300), to identify other proteins that bind to or interact with PPI ("PPI-binding proteins" or "PPI-bp") and modulate PPI activity. Such PPI-binding proteins are also likely to be involved in the propagation of signals by the PPI proteins as, for example, upstream or downstream elements of the PPI pathway.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for PPI is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming a PPI-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) that is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene that encodes the protein which interacts with PPI.

In yet another aspect of the invention are methods which utilize the transgenic plants of the invention to identify PPI-interacting components via genetic screening protocols. These components can be for example, regulatory elements which modify PPI-gene expression, interacting proteins which directly modify PPI activity or interacting proteins which modify components of the same signal transduction pathway and thereby exert an effect on the expression or activity of PPI. Briefly, genetic screening protocols are applied to the transgenic plants of the invention and in so doing identify related genes which are not identified using a wild type background for the screen. For example an activation tagged library (Weigel, *et al.*, 2000. *Plant Physiol.* 122: 1003-1013), can be produced using the transgenic plants of the invention as the genetic background. Plants are then screened for altered phenotypes from that displayed by the parent plants. Alternative methods of generating libraries from the transgenic plants of the invention can be used, for example, chemical or irradiation induced mutations, insertional inactivation or insertional activation methods.

The invention further pertains to novel agents identified by the aforementioned screening assays and uses thereof.

Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding a PPI protein, or derivatives, fragments, analogs or homologs thereof. As used herein, the term "vector" refers to a nucleic acid molecule capable of

transporting another nucleic acid to which it has been linked. Exemplary expression vector constructs include for example the constructs of SEQ ID NO: 54-64. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication). Other vectors are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively-linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors or plant transformation vectors, binary or otherwise, which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, that is operatively-linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably-linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner that allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell).

The term "regulatory sequence" is intended to includes promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell and those that direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). Examples of suitable promoters include for example constitutive promoters, ABA inducible promoters, tissue specific promters or guard cell specific promoters. It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced

into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (*e.g.*, PPI proteins, mutant forms of PPI proteins, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of PPI proteins in prokaryotic or eukaryotic cells. For example, PPI proteins can be expressed in bacterial cells such as *Escherichia coli*, insect cells (using baculovirus expression vectors) yeast cells, plant cells or mammalian cells. Suitable host cells are discussed further in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *Escherichia coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: (*i*) to increase expression of recombinant protein; (*ii*) to increase the solubility of the recombinant protein; and (*iii*) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson, 1988. *Gene* 67: 31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) that fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amrann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 60-89).

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein. *See, e.g.,* Gottesman, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 119-128. Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual

codons for each amino acid are those preferentially utilized in *E. coli* (*see, e.g.,* Wada, *et al.*, 1992. *Nucl. Acids Res.* 20: 2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the PPI expression vector is a yeast expression vector. Examples of vectors for expression in yeast *Saccharomyces cerivisae* include pYepSec1 (Baldari, *et al.*, 1987. *EMBO J.* 6: 229-234), pMFa (Kurjan and Herskowitz, 1982. *Cell* 30: 933-943), pJRY88 (Schultz *et al.*, 1987. *Gene* 54: 113-123), pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (InVitrogen Corp, San Diego, Calif.).

Alternatively, PPI can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (*e.g.*, SF9 cells) include the pAc series (Smith, *et al.*, 1983. *Mol. Cell. Biol.* 3: 2156-2165) and the pVL series (Lucklow and Summers, 1989. *Virology* 170: 31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, 1987. *Nature* 329: 840) and pMT2PC (Kaufman, *et al.*, 1987. *EMBO J.* 6: 187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, adenovirus 2, cytomegalovirus, and simian virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see, *e.g.*, Chapters 16 and 17 of Sambrook, *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

In yet another embodiment, a nucleic acid of the invention is expressed in plants cells using a plant expression vector. Examples of plant expression vectors systems include tumor inducing (Ti) plasmid or portion thereof found in *Agrobacterium*, cauliflower mosaic virus (CAMV) DNA and vectors such as pBI121 .

For expression in plants, the recombinant expression cassette will contain in addition to the PPI nucleic acids, a plant promoter region, a transcription initiation site (if the coding sequence to transcribed lacks one), and a transcription termination/polyadenylation sequence. The termination/polyadenylation region may be obtained from the same gene as the promoter sequence or may be obtained from different genes. Unique restriction enzyme sites at the 5' and 3' ends of the cassette are typically included to allow for easy insertion into a pre-existing vector. Examples of suitable promotors include promoters from plant viruses such as the 35S promoter from cauliflower mosaic virus (CaMV). Odell, et al., Nature, 313: 810-812 (1985). and promoters from genes such as rice actin (McElroy, et al., Plant Cell, 163-171 (1990)); ubiquitin

(Christensen, et al., Plant Mol. Biol., 12: 619-632 (1992); and Christensen, et al., Plant Mol. Biol., 18: 675-689 (1992)); pEMU (Last, et al., Theor. Appl. Genet., 81: 581-588 (1991)); MAS (Velten, et al., EMBO J., 3: 2723-2730 (1984)); maize H3 histone (Lepetit, et al., Mol. Gen. Genet., 231: 276-285 (1992); and Atanassvoa, et al., Plant Journal, 2(3): 291-300 (1992)), the 5'- or 3'-promoter derived from T-DNA of *Agrobacterium* tumefaciens, the Smas promoter, the cinnamyl alcohol dehydrogenase promoter (U.S. Pat. No. 5,683,439), the Nos promoter, the rubisco promoter, the GRP1-8 promoter, ALS promoter, (WO 96/30530), a synthetic promoter, such as, Rsyn7, SCP and UCP promoters, ribulose-1,3-diphosphate carboxylase, fruit-specific promoters, heat shock promoters, seed-specific promoters and other transcription initiation regions from various plant genes, for example, include the various opine initiation regions, such as for example, octopine, mannopine, and nopaline.

Additional regulatory elements that may be connected to a PPI encoding nucleic acid sequence for expression in plant cells include terminators, polyadenylation sequences, and nucleic acid sequences encoding signal peptides that permit localization within a plant cell or secretion of the protein from the cell. Such regulatory elements and methods for adding or exchanging these elements with the regulatory elements PPI gene are known, and include, but are not limited to, 3' termination and/or polyadenylation regions such as those of the *Agrobacterium* tumefaciens nopaline synthase (nos) gene (Bevan, et al., Nucl. Acids Res., 12: 369-385 (1983)); the potato proteinase inhibitor II (PINII) gene (Keil, et al., Nucl. Acids Res., 14: 5641-5650 (1986) and hereby incorporated by reference); and An,, et al., Plant Cell, 1: 115-122 (1989)); and the CaMV 19S gene (Mogen, et al., Plant Cell, 2: 1261-1272 (1990)).

Plant signal sequences, including, but not limited to, signal-peptide encoding DNA/RNA sequences which target proteins to the extracellular matrix of the plant cell (Dratewka-Kos, et al., J. Biol. Chem., 264: 4896-4900 (1989)) and the *Nicotiana plumbaginifolia* extension gene (DeLoose, et al., Gene, 99: 95-100 (1991)), or signal peptides which target proteins to the vacuole like the sweet potato sporamin gene (Matsuka, et al., Proc. Nat'l Acad. Sci. (USA), 88: 834 (1991)) and the barley lectin gene (Wilkins, et al., Plant Cell, 2: 301-313 (1990)), or signals which cause proteins to be secreted such as that of PRIb (Lind, et al., Plant Mol. Biol., 18: 47-53 (1992)), or those which target proteins to the plastids such as that of rapeseed enoyl-ACP reductase (Verwaert, et al., Plant Mol. Biol., 26: 189-202 (1994)) are useful in the invention.

In another embodiment, the recombinant expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements

are known in the art. Especially useful in connection with the nucleic acids of the present invention are expression systems which are operable in plants. These include systems which are under control of a tissue-specific promoter, as well as those which involve promoters that are operable in all plant tissues.

Organ-specific promoters are also well known. For example, the patatin class I promoter is transcriptionally activated only in the potato tuber and can be used to target gene expression in the tuber (Bevan, M., 1986, *Nucleic Acids Research* 14:4625-4636). Another potato-specific promoter is the granule-bound starch synthase (GBSS) promoter (Visser, R.G.R, *et al.*, 1991, *Plant Molecular Biology* 17:691-699).

Other organ-specific promoters appropriate for a desired target organ can be isolated using known procedures. These control sequences are generally associated with genes uniquely expressed in the desired organ. In a typical higher plant, each organ has thousands of mRNAs that are absent from other organ systems (reviewed in Goldberg, P., 1986, *Trans. R. Soc. London* B314:343).

For in situ production of the antisense mRNA of GST, those regions of the GST gene which are transcribed into GST mRNA, including the untranslated regions thereof, are inserted into the expression vector under control of the promoter system in a reverse orientation. The resulting transcribed mRNA is then complementary to that normally produced by the plant.

The resulting expression system or cassette is ligated into or otherwise constructed to be included in a recombinant vector which is appropriate for plant transformation. The vector may also contain a selectable marker gene by which transformed plant cells can be identified in culture. Usually, the marker gene will encode antibiotic resistance. These markers include resistance to G418, hygromycin, bleomycin, kanamycin, and gentamicin. After transforming the plant cells, those cells having the vector will be identified by their ability to grow on a medium containing the particular antibiotic. Replication sequences, of bacterial or viral origin, are generally also included to allow the vector to be cloned in a bacterial or phage host, preferably a broad host range prokaryotic origin of replication is included. A selectable marker for bacteria should also be included to allow selection of bacterial cells bearing the desired construct. Suitable prokaryotic selectable markers also include resistance to antibiotics such as kanamycin or tetracycline.

Other DNA sequences encoding additional functions may also be present in the vector, as is known in the art. For instance, in the case of *Agrobacterium* transformations, T-DNA sequences will also be included for subsequent transfer to plant chromosomes.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell.

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) a polypeptide of the invention encoded in a an open reading frame of a polynucleotide of the invention. Accordingly, the invention further provides methods for producing a polypeptide using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding a polypeptide of the invention has been introduced) in a suitable medium such that the polypeptide is produced. In another embodiment, the method further comprises isolating the polypeptide from the medium or the host cell.

A number of types of cells may act as suitable host cells for expression of a polypeptide encoded by an open reading frame in a polynucleotide of the invention. Plant host cells include, for example, plant cells that could function as suitable hosts for the expression of a polynucleotide of the invention include epidermal cells, mesophyll and other ground tissues, and vascular tissues in leaves, stems, floral organs, and roots from a variety of plant species, such as *Arabidopsis thaliana, Nicotiana tabacum, Brassica napus, Zea mays, Oryza sativa, Gossypium hirsutum and Glycine max.*

Alternatively, it may be possible to produce a polypeptide in lower eukaryotes such as yeast or in prokaryotes such as bacteria. Potentially suitable yeast strains include *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Kluyveromyces* strains, *Candida,* or any yeast strain capable of expressing heterologous proteins. Potentially suitable bacterial strains include *Escherichia coli, Bacillus subtilis, Salmonella typhimurium*, or any bacterial strain capable of expressing heterologous polypeptides. If the polypeptide is made in yeast or bacteria, it may be necessary to modify the polypeptide produced therein, for example by phosphorylation or glycosylation of the appropriate sites, in order to obtain a functional

polypeptide, if the polypeptide is of sufficient length and conformation to have activity. Such covalent attachments may be accomplished using known chemical or enzymatic methods.

A polypeptide may be prepared by culturing transformed host cells under culture conditions suitable to express the recombinant protein. The resulting expressed polypeptide or protein may then be purified from such culture (e.g., from culture medium or cell extracts) using known purification processes, such as gel filtration and ion exchange chromatography. The purification of the polypeptide or protein may also include an affinity column containing agents which will bind to the protein; one or more column steps over such affinity resins as concanavalin A-agarose, heparin-toyopearl® or Cibacrom blue 3GA Sepharose®; one or more steps involving hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or immunoaffinity chromatography.

Alternatively, a polypeptide or protein may also be expressed in a form which will facilitate purification. For example, it may be expressed as a fusion protein containing a six-residue histidine tag. The histidine-tagged protein will then bind to a Ni-affinity column. After elution of all other proteins, the histidine-tagged protein can be eluted to achieve rapid and efficient purification. One or more reverse-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify a polypeptide. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a substantially homogeneous isolated recombinant polypeptide. The protein or polypeptide thus purified is substantially free of other plant proteins or polypeptides and is defined in accordance with the present invention as "isolated."

Transformed Plants Cells and Transgenic Plants

The invention includes protoplast, plants cells, plant tissue and plants (*e.g.,* monocots and dicots transformed with a PPI nucleic acid (*i.e,* sense or antisense), a vector containing a PPI nucleic acid (*i.e,* sense or antisense)or an expression vector containing a PPI nucleic acid (*i.e,* sense or antisense). As used herein, "plant" is meant to include not only a whole plant but also a portion thereof (*i.e.,* cells, and tissues, including for example, leaves, stems, shoots, roots, flowers, fruits and seeds).

The plant can be any plant type including, for example, species from the genera *Cucurbita, Rosa, Vitis, Juglans, Fragaria, Lotus, Medicago, Onobrychis, Trifolium, Trigonella, Vigna, Citrus, Linum, Geranium, Manihot, Daucus, Arabidopsis, Brassica, Raphanus, Sinapis,*

Atropa, Capsicum, Datura, Hyoscyamus, Lycopersicon, Nicotiana, Solanum, Petunia, Digitalis, Majorana, Ciahorium, Helianthus, Lactuca, Bromus, Asparagus, Antirrhinum, Heterocallis, Nemesis, Pelargonium, Panieum, Pennisetum, Ranunculus, Senecio, Salpiglossis, Cucumis, Browaalia, Glycine, Pisum, Phaseolus, Lolium, Oryza, Zea, Avena, Hordeum, Secale, Triticum, Sorghum, Gossypium, Picea, Caco, and *Populus*.

In some aspects of the invention, the transformed plant is resistant to biotic and abiotic stresses, *e.g.*, chilling stress, salt stress, water stress (e.g., drought), disease, grazing pests and wound healing. Additionally, the invention also includes a transgenic plant that is resistant to pathogens such as for example fungi, bacteria, nematodes, viruses and parasitic weeds. Alternatively, the transgenic plant is resistant to herbicides or has delayed senesence. The transgenic plant has an increase in yield, productivity, biomass or ABA sensitivity. By resistant is meant the plant grows under stress conditions (*e.g.*, high salt, decreased water, low temperatures) or under conditions that normally inhibit, to some degree, the growth of an untransformed plant. Methodologies to determine plant growth or response to stress include for example, height measurements, weight meaurements, leaf area, ability to flower, water use, transpiration rates and yield.

The invention also includes cells, tissues, including for example, leaves, stems, shoots, roots, flowers, fruits and seeds and the progeny derived from the tranformed plant.

Numerous methods for introducing foreign genes into plants are known and can be used to insert a gene into a plant host, including biological and physical plant transformation protocols. See, for example, Miki et al., (1993) "Procedure for Introducing Foreign DNA into Plants", In: Methods in Plant Molecular Biology and Biotechnology, Glick and Thompson, eds., CRC Press, Inc., Boca Raton, pages 67-88 and Andrew Bent in, Clough SJ and Bent AF, 1998. Floral dipping: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*.. The methods chosen vary with the host plant, and include chemical transfection methods such as calcium phosphate, polyethylene glycol (PEG) transformation, microorganism-mediated gene transfer such as *Agrobacterium* (Horsch, et al., Science, 227: 1229-31 (1985)), electroporation, protoplast transformation, micro-injection, flower dipping and biolistic bombardment.

Agrobacterium-mediated Transformation

The most widely utilized method for introducing an expression vector into plants is based on the natural transformation system of *Agrobacterium*. *A. tumefaciens* and *A. rhizogenes*

are plant pathogenic soil bacteria which genetically transform plant cells. The Ti and Ri plasmids of *A. tumefaciens* and *A. rhizogenes*, respectfully, carry genes responsible for genetic transformation of plants. See, for example, Kado, Crit. Rev. Plant Sci., 10: 1-32 (1991). Descriptions of the *Agrobacterium* vector systems and methods for *Agrobacterium*-mediated gene transfer are provided in Gruber et al., supra; and Moloney, et al, Plant Cell Reports, 8: 238-242 (1989).

Transgenic *Arabidopsis* plants can be produced easily by the method of dipping flowering plants into an *Agrobacterium* culture, based on the method of Andrew Bent in, Clough SJ and Bent AF, 1998. Floral dipping: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. Wild type plants are grown until the plant has both developing flowers and open flowers. The plant are inverted for 1 minute into a solution of *Agrobacterium* culture carrying the appropriate gene construct. Plants are then left horizontal in a tray and kept covered for two days to maintain humidity and then righted and bagged to continue growth and seed development. Mature seed is bulk harvested.

Direct Gene Transfer

A generally applicable method of plant transformation is microprojectile-mediated transformation, where DNA is carried on the surface of microprojectiles measuring about 1 to 4 mu.m. The expression vector is introduced into plant tissues with a biolistic device that accelerates the microprojectiles to speeds of 300 to 600 m/s which is sufficient to penetrate the plant cell walls and membranes. (Sanford, et al., Part. Sci. Technol., 5: 27-37 (1987); Sanford, Trends Biotech, 6: 299-302 (1988); Sanford, Physiol. Plant, 79: 206-209 (1990); Klein, et al., Biotechnology, 10: 286-291 (1992)).

Another method for physical delivery of DNA to plants is sonication of target cells as described in Zang, et al., BioTechnology, 9: 996-996 (1991). Alternatively, liposome or spheroplast fusions have been used to introduce expression vectors into plants. See, for example, Deshayes, et al., EMBO J., 4: 2731-2737 (1985); and Christou, et al., Proc. Nat'l. Acad. Sci. (USA), 84: 3962-3966 (1987). Direct uptake of DNA into protoplasts using CaCl.sub.2 precipitation, polyvinyl alcohol or poly-L-ornithine have also been reported. See, for example, Hain, et al., Mol. Gen. Genet., 199: 161 (1985); and Draper, et al., Plant Cell Physiol., 23: 451-458 (1982).

Electroporation of protoplasts and whole cells and tissues has also been described. See, for example, Donn, et al., (1990) In: Abstracts of the VIIth Int;l. Congress on Plant Cell and Tissue Culture IAPTC, A2-38, page 53; D'Halluin et al., Plant Cell, 4: 1495-1505 (1992); and Spencer et al., Plant Mol. Biol., 24: 51-61 (1994).

Particle Wounding/Agrobacterium Delivery

Another useful basic transformation protocol involves a combination of wounding by particle bombardment, followed by use of *Agrobacterium* for DNA delivery, as described by Bidney, et al., Plant Mol. Biol., 18: 301-31 (1992). Useful plasmids for plant transformation include Bin 19. See Bevan, Nucleic Acids Research, 12: 8711-8721 (1984), and hereby incorporated by reference.

In general, the intact meristem transformation method involves imbibing seed for 24 hours in the dark, removing the cotyledons and root radical, followed by culturing of the meristem explants. Twenty-four hours later, the primary leaves are removed to expose the apical meristem. The explants are placed apical dome side up and bombarded, e.g., twice with particles, followed by co-cultivation with *Agrobacterium*. To start the co-cultivation for intact meristems, *Agrobacterium* is placed on the meristem. After about a 3-day co-cultivation period the meristems are transferred to culture medium with cefotaxime plus kanamycin for the NPTII selection.

The split meristem method involves imbibing seed, breaking of the cotyledons to produce a clean fracture at the plane of the embryonic axis, excising the root tip and then bisecting the explants longitudinally between the primordial leaves. The two halves are placed cut surface up on the medium then bombarded twice with particles, followed by co-cultivation with *Agrobacterium*. For split meristems, after bombardment, the meristems are placed in an *Agrobacterium* suspension for 30 minutes. They are then removed from the suspension onto solid culture medium for three day co-cultivation. After this period, the meristems are transferred to fresh medium with cefotaxime plus kanamycin for selection.

Transfer by Plant Breeding

Alternatively, once a single transformed plant has been obtained by the foregoing recombinant DNA method, conventional plant breeding methods can be used to transfer the gene and associated regulatory sequences via crossing and backcrossing. Such intermediate methods will comprise the further steps of: (1) sexually crossing the transgenic plant with a plant from a second taxon; (2) recovering reproductive material from the progeny of the cross; and (3) growing transgenic plants from the reproductive material. Where desirable or necessary, the agronomic characteristics of the second taxon can be substantially preserved by expanding this method to include the further steps of repetitively: (1) backcrossing the transgenic progeny with non-transgenic plants from the second taxon; and (2) selecting for expression of an associated

marker gene among the progeny of the backcross, until the desired percentage of the characteristics of the second taxon are present in the progeny along with the gene or genes imparting marker gene trait.

By the term "taxon" herein is meant a unit of botanical classification. It thus includes, genus, species, cultivars, varieties, variants and other minor taxonomic groups which lack a consistent nomenclature.

Regeneration of Transformants

The development or regeneration of plants from either single plant protoplasts or various explants is well known in the art (Weissbach and Weissbach, 1988). This regeneration and growth process typically includes the steps of selection of transformed cells, culturing those individualized cells through the usual stages of embryonic development through the rooted plantlet stage. Transgenic embryos and seeds are similarly regenerated. The resulting transgenic rooted shoots are thereafter planted in an appropriate plant growth medium such as soil.

The development or regeneration of plants containing the foreign, exogenous gene that encodes a polypeptide of interest introduced by *Agrobacterium* from leaf explants can be achieved by methods well known in the art such as described (Horsch et al., 1985). In this procedure, transformants are cultured in the presence of a selection agent and in a medium that induces the regeneration of shoots in the plant strain being transformed as described (Fraley et al., 1983). In particular, U.S. Pat. No. 5,349,124 (specification incorporated herein by reference) details the creation of genetically transformed lettuce cells and plants resulting therefrom which express hybrid crystal proteins conferring insecticidal activity against Lepidopteran larvae to such plants.

This procedure typically produces shoots within two to four months and those shoots are then transferred to an appropriate root-inducing medium containing the selective agent and an antibiotic to prevent bacterial growth. Shoots that rooted in the presence of the selective agent to form plantlets are then transplanted to soil or other media to allow the production of roots. These procedures vary depending upon the particular plant strain employed, such variations being well known in the art.

Preferably, the regenerated plants are self-pollinated to provide homozygous transgenic plants, or pollen obtained from the regenerated plants is crossed to seed-grown plants of agronomically important, preferably inbred lines. Conversely, pollen from plants of those important lines is used to pollinate regenerated plants. A transgenic plant of the present

invention containing a desired polypeptide is cultivated using methods well known to one skilled in the art.

A preferred transgenic plant is an independent segregant and can transmit the gene and its activity to its progeny. A more preferred transgenic plant is homozygous for the gene, and transmits that gene to all of its offspring on sexual mating. Seed from a transgenic plant may be grown in the field or greenhouse, and resulting sexually mature transgenic plants are self-pollinated to generate true breeding plants. The progeny from these plants become true breeding lines that are evaluated for increased expression of the transgene.

EMBODIMENTS

The constructs and methods of this invention have numerous applications of commercial value, especially in the prevention of desiccation of plant tissues under periods of water stress. Genetic manipulation of crop plants incorporating inhibitors of Ftase or inactivation of the gene encoding endogenous plant Ftase would allow such plants to withstand transitory environmental stress and can broaden the environments where these plants can be grown. Thus, improving tolerance of crop plants to cold, salt and drought stress, can improve the yield of the plants under such adverse conditions.

The technology described herein can also be used to alter harvesting time and harvest quality of plants. For example, overexpression of Ftase could lead to faster drying times of crops, such as corn and other grasses. Drying corn involves the use of large amounts of propane gas. Drying times of crops such as hay, which dry naturally in the fields, could be shortened, making it less likely that rain would deteriorate the crop.

In addition, inhibition of farnesylation in plants can also be used to control the senescence program of the plants so that leaves can be maintained in a green state longer and fruits can be kept immature. For example, if an antisense construct of ERA1 or CaaX box inhibitor protein construct was placed under the control of a senescence-induced promoter, the plant would induce an inhibitor of farnesylation as the senescence program was initiated, which would in turn inhibit senescence. The result would be a plant which remains green or fruits which remain immature. Thus, the plant could be kept producing a product, such as a vegetative part, flower or fruit much longer. Thus, horticulturalists could produce plants which stayed green and continued to grow even though a wild-type plant of the same variety would senesce under the same conditions. Cut flowers could be maintained longer. Or a fruit could be kept

immature, an important product for the vegetable industry where produce lifetime to market is extremely important.

Further, the inhibition of Ftase in fruits and vegetables can reduce wilting. Thus, wilting of produce during transport and shipping could be reduced. Fruits and vegetables on the grocery shelf would also require less misting to keep them fresh and flavorful, and there would be less need to wax produce such as cucumber, apples and oranges to keep them from drying out.

Less watering would also mean that fungal and bacterial attacks on the crops, or fruits and vegetables would be reduced. For example, plant diseases in the field which result from splashing of plant pathogens from the soil to the plant leaves and fruits could be inhibited.

In the field of horticulture, many drought-resistant varieties could be produced for landscaping and for use as ornamental house plants. Especially valuable would be varieties of plants which are used for potting, as ornamentals inside or outside homes and offices, and which can survive infrequent water. This would be a considerable boon for gardeners, especially during the droughty summer months where forgotten plants dry out quickly in the sun. Further, plants grown under trees and in other shady areas often experience drought conditions and limited light. The technology provided herein can provide plant varieties which can better survive under these conditions.

In a further embodiment, horitculturalists could find many uses for plants wherein lateral branching and/or flower numbers can be regulated with light/dark cycles. Examples of plants in which longer, unbranched stems would confer marketable advantage include roses, carnations, lilies, and the like. The ability to increase the number of flowers or florets on the plant is also a highly valuable asset. These traits could also be useful for many agricultural crops in that yields can be increased in a manner which also made harvesting of the crop easier.

Another benefit of the constructs and methods provided herein is that the ERA1 promoter is active in the guard cells of leaves. A portion of the ERA1 gene promoter can be fused to antisense nucleic acid to the ERA1 gene so Ftase activity is diminished only in the guard cells.

A further embodiment is the use of the drought-resistant trait as a selectable marker of transformation in plants, plant cells and plant tissues. One method of detecting transformation in plants consists of: (a) incorporating a nucleic acid construct comprising a promoter operably-linked to nucleic acid comprising antisense to SEQ ID NO:1 or nucleic acid comprising a functional equivalent or fragment thereof of the antisense; (b) inserting the nucleic acid construct into a plant, plant cell or plant tissue; (c) growing the plant, or regenerating a plant from the plant cell or plant tissue until stomates are formed; and (d) placing the plant or

regenerated plant under conditions wherein the plant is drought stressed, wherein survival of the plant under drought conditions compared to untransformed plants is indicative of transformation. Thus, this technology can be used as a selectable genetic marker, *i.e.*, a visual marker especially when combined with plant selection and transformation schemes.

In addition, without resorting to stressing a transgenic plant, the branching and/or flowering habit of plants with loss of Ftase function differs substantially from that of wild-type plants and can be used as a marker for successful transformation. This method would be especially useful where *in planta* transformation techniques have been applied. Under diurnal light conditions, shoots of transgenic plants will demonstrate less lateral branching than that of untransformed shoots, thus indicating effective loss of Ftase activity without the use of selective antibiotic markers.

EXEMPLIFICATION

Example 1: Mutagenesis conditions

Arabidopsis plants used in this study were grown under continuous light in soil- or agar-containing petri plates as described elsewhere (Haughn and Somerville 1986). Two distinct wild-types of *Arabidopsis* were used: Meyerowitz's Colombia (MCol) (Lelhe Seeds, Dripping Springs, TX) and Wassilewskija (Ws) (ABRC, Ohio State University). T-DNA mutagenized seeds were screened and mutants were isolated in the Wassilewskija background. These were obtained from the Ohio State *Arabidopsis* seed stock collection (ABRC stock numbers CS2606-2654). The T-DNA seed collection was comprised of 49 pools of 1200 fourth generation (T4) offspring derived from 100 mutagenized parents. A mutagenized parent was obtained by incubating wild-type (T1) seeds overnight in a saturating *Agrobacterium* culture containing a T-DNA plasmid carrying a gene conferring kanamycin resistance. The seeds were then washed in water and planted into pots. T2 generation seed were obtained from each plant and tested for kanamycin resistance. Kanamycin-resistant plants were advanced to the T3 generation. T4 generation plants were given to the stock center. Each pool was screened separately.

Fast neutron-irradiated seeds were screened and mutants were isolated in Meyerowitz's Columbia background. Mutagenized wild-type seeds (N1) were irradiated with 60 Gy of fast neutrons and grown to the next generation. The N2 seeds were obtained as pools of

approximately 11,000 seeds generated from 1387 N1 parents. Ten of these pools were screened separately for ABA supersensitive mutations. In the initial screen, all seeds had been stored at 4°C and were plated without imbibing. For all subsequent screens, seeds were imbibed at 4°C for one week on 0.3 μM ABA and scored for cotyledon emergence after 5-7 days at 22°C in the light.

Example 2: Genetic Analysis

Mutant lines were backcrossed to wild type three times. T-DNA mutations were backcrossed to Ws and fast neutron mutants to MCol. Segregation of the *era* phenotype was followed by plating F2 seeds on both 0.3 μM ABA and imbibing four days at 4°C. Following imbibition, plates were transferred to room temperature in the light. Germination was measured as the presence or absence of expanded cotyledons in seedlings one week after imbibition. Double mutants were constructed by crossing lines homozygous for each mutation following segregation and identifying lines that carried one of the mutant phenotypes. The *abi*3 allele used in this study is *abi*3-6 (Nambara *et al.*, 1994) and the *abi*1 allele is *abi*1-1 (Koornneef *et al.*, 1982). The *era*1-2 allele was used as the *era* parent. Segregation analysis suggested *era*1 partially suppressed the insensitivity of *abi*1 to ABA, so F2 plants were first screened for insensitivity to 3 mM ABA, and F3 seed from these plants were scored for sensitivity to 0.3 μM ABA. Putative *era1 abi1* double mutants were progeny-tested in the F4 generation and verified by DNA polymorphism analysis for both Era 1 and Abi1. For *era1 abi3* double mutants, F2 seeds were screened for insensitivity to 3 μM ABA, and mature plants were scored for protruding carpels and immature green seeds (Nambara *et al.*, 1994). Putative double mutant lines were also verified by DNA polymorphism analysis for both Era1 and Abi3.

Example 3: DNA and RNA Analysis

The methods employed for DNA (Dellaporta *et al.*, 1983) and RNA (Verwoerd *et al.*, 1989) extractions were as described elsewhere. High stringency Southern blots were carried out at 65°C according to standard protocols described elsewhere (Sambrook *et al.*, 1989). All genomic and cDNA library screening was done on Gelman BioTrace NT membranes according to the manufacturer's specifications (Gelman Sciences). To clone insertion junctions between T-DNA and genomic DNA in the *era1-1* mutant (isolated from T12W DNA) a library of T12W DNA was made in γ-ZAPII (Stratagene). Genomic Southern blots of T12W DNA digested with restriction endonuclease *Eco*R I and probed with right border (RB) T-DNA produced three

bands (13.0 Kb, 7.0 Kb and 8.0 Kb). Subsequent analysis with additional restriction enzymes verified that the 7.0 and 8.0 Kb bands contained the insertion junctions between T-DNA and flanking plant DNA. These fragments were cloned by digesting genomic DNA with *Eco*R I, fractionating the digested DNA using a Prep Cell (Pharmacia), and identifying the fractions containing the 7.0 and 8.0 Kb by Southern blot analysis using the RB as a probe. Pooled fractions containing both the 7.0 and 8.0 Kb fragments were then ligated to the γ-ZAPII vector arms according to the manufacturer's instructions (Stratagene). A library containing approximately 40,000 individual recombinant bacteriophage was screened. Five positive plaques were identified and excised plasmid forms of the cloned inserts were isolated according to the manufacturer's instructions (Stratagene). Two plasmids which hybridized to the RB probe were designated pL4B and pL7 and selected for further characterization. A 2.3 kB *Eco*R I-*Bam*H I restriction fragment from clone pL4B was subcloned into the plasmid pBluescript and designated pSC10. A 1.3 Kb *Hin*d III- *Bam*H I restriction fragment from clone pL7 was also subcloned into pBluescript and designated pSC11. Each of these plasmids contains approximately 1.2 Kb of T-DNA attached to the flanking plant genomic DNA. pSC10 was used as a probe to screen an *Arabidopsis* cDNA library called PRL2 λ-ZipLox (ABRC, Stock CD4-7). This screen identified five positive cDNAs, and the longest cDNA insert, clone pZL23, was used to screen an additional 200,000 recombinant PRL2 phage. Subsequently a longer cDNA insert, clone pZL51, which contained an insert of 1.35 Kb, was isolated. Both cDNA clones pZL23 and pZL51 were sequenced and used to screen 30,000 γ-ZAPII plaques made from wild-type Columbia genomic DNA partially digested with *Eco*R I. Construction of this library was as described above except the digested DNA was not size-fractionated. This screen identified four positive clones. The inserts were excised and excised plasmid forms of the cloned inserts were isolated according to the manufacturer's instructions. A 6 Kb region encompassing the entire pZL51 clone was completely sequenced. This genomic insert and a 14 Kb genomic insert isolated by screening a λ-FIX genomic library from *Lansberg erecta* via similar methods (ABRC Stock CD4-8) were used as probes to analyze deletion size in the fast neutron mutants *era*1-2 and *era*1-3.

Example 4: Protein Farnesyl Transferase Assay

Farnesyl transferase (Ftase) assays were performed using Ftase from cell-free extracts of wild-type and mutant plants and synthetic heptapeptides as substrate for the reaction. Peptides were purchased from Genemed Biotechnologies, Inc. The peptide sequences used were based

on the data of Randall *et al.* (1993): GGCCAIM (-CAIM) and GGCCAIL(-CAIL). Solutions of peptides were prepared in 100% dimethyl sulfoxide (DMSO) containing 10 mM dithiotreitol (DTT) and diluted in 10 mM DTT without DMSO. The cell-free extracts contained soluble protein isolated from the buds of three week old plants, either wild-type or mutant strains. First 1 g of fresh buds was collected and homogenized in a buffer containing 50 mM Hepes (pH 7.5), 1 mM $MgCl_2$, 1 mM EGTA, 5 mM DTT, 2 μg/ml leupeptin, 2 μg/ml aprotinin, and 1 mM PMSF. Next, cellular debris and membranes were removed by centrifugation at 4°C at 10,000×g for 10 minutes and 100,000×g for 30 minutes. Following the second centrifugation, the supernatant was decanted and total soluble protein was quantified by the method of Bradford (1976). Soluble protein extracts were incubated at 30°C with a peptide substrate and radio-labeled ^{3}H-farnesyl pyrophosphate (FPP) (Amersham) for 40 minutes. Each reaction mixture contained the following components in a final volume of 25 μl: 50 mM Hepes (pH 7.5), 5 mM $MgCl_2$, 5 mM DTT, 50 μM peptide, 0.5 μM [^{3}H]FPP, and 100 μg of soluble protein extract. One control reaction contained soluble protein extracts that had been boiled for 5 minutes to irreversibly denature all protein. Reactions were terminated by adding EDTA to a final concentration of 50 mM and then spotted onto Silica Gel 60 thin-layer chromatography (TLC) plates (Millipore). TLC plates were developed with *n*-propanol and water (7:3 v/v) for 4-5 hours. The plates were dried, sprayed with En^{3}Hance (New England Nuclear), and exposed to Kodak X-OMAT AR film at -70°C for 4 days.

Example 5: ERA1-β-glucuronidase gene constructs and transgenic plants

ERA1-β-glucuronidase (ERA1-GUS) fusion constructs were made by inserting a 5 Kb *Eco*R I-*Hin*d III genomic fragment of the ERA1 promoter into a promoterless GUS T-DNA plasmid pBI121 containing a gene conferring resistance to the antibiotic ampicillin. This construct was then transformed into *Agrobacterium* strain LB4404. The *Agrobacterium* was grown to a density of 0.8 O.D. units (measured at 595 nm). The cells were then washed extensively in water, resuspended in sterile 10% glycerol and purified plasmid DNA encoding the ERA1-GUS fusion construct was added. Finally, the mixture of cells and DNA was pulsed in an electroporator at 200 Ohms 25 μF, 2.5 kvolts. Cells were then plated on Luria Broth agar plates containing 100 μg/ml ampicillin and grown for 2 days at 28°C. Ampicillin-resistant transformants were cultured and plasmid DNA isolated from the cultures by standard techniques was used in subsequent plant transformation experiments.

Transgenic plants were made by vacuum infiltrating plants with a saturated *Agrobacterium* culture grown to a density of 0.8 O.D. units as measured at 595 nm. Wild-type plants were grown under standard laboratory conditions (at 25°C, 150 μE m^{-2} sec^{-1}, humidity, constant light) until they produced their first bolts at approximately 5 weeks. Next, plant stems were removed and the plants were submerged in a solution of *Agrobacterium* and placed under a 20 mBar vacuum for 5 minutes. After the vacuum was broken, the plants were transferred to soil and allowed to recover under standard laboratory conditions as described above. After two months, the plants produced new flowers and seed which was harvested and allowed to dry for 2 weeks. Seed from individual plants were planted onto Murashige and Skoog (MS) minimal medium plates containing 50 μg/ml kanamycin. Green kanamycin-resistant plantlets were identified and transferred to soil after 2 weeks and allowed to grow for seed. These seeds were germinated and the seedlings were tested for GUS activity using the fluorescent GUS substrate Imagene Green (Molecular Probes, Eugene, Oregon). GUS activity was assayed by suspending seedlings in GUS buffer (50 mM Sodium phosphate, pH 7.0, 10 mM EDTA, 0.1% Triton X-100, 0.1% Sodium sarcosyl, 4 mM Imagene Green) for 2-4 hours in the dark at room temperature. Seedlings were viewed under a microscope at 25X magnification using blue light to generate a positive fluorescent signal. When this mixture is treated with blue light, GUS activity will produce yellow light in a background of red auto-fluorescence generated by red chlorophyll.

Example 6: Drought Experiments

Six wild-type and six *era*1-2 seedlings were grown for four weeks in constant light with constant watering (25°C, 150 μE m^{-2} sec^{-1}, 70% humidity, constant light). The plant and pot were weighed and the pots were then covered with aluminum foil to retard soil evaporation. At this time, plants were no longer watered and each pot was weighed daily. At the end of the experiment plants were removed from the pots, which were allowed to dry for another two weeks, when they were weighed to determine the weight of the dry soil and pot. This weight was subtracted from each sample.

Example 7: Age-related changes in detached leaves

The chlorophyll content in adult rosette leaves in wild-type Columbia and *era*1-2 mutants were compared after detachment from plants. The plants were grown under constant light and temperature (150 μE/m^2·sec, 22°C) to a similar developmental age of 3 weeks after germination. At this time, the fifth leaves of several plants which had emerged after germination

were removed and placed on petri plates containing 0.8% agar with minimal salts. The plates were sealed and placed at 22°C under constant light (50 $\mu E/m^2 \cdot sec$) for 12 days. Photographs were taken and color comparisons made at 0, 3, 6, 9, and 12 days.

Example 8: Determination of transcript levels for selected genes in aging leaves.

Mutant (*era1-2*) and wild-type plants were grown under constant light and temperature (150 $\mu E/m^2 \cdot sec$, 22°C) to a similar developmental age of 4 weeks after germination. At that time, the fifth rosette leaf which had emerged following germination was removed from all plants. These leaves were assayed for expression levels of three genes: *Arabidopsis* chlorophyll binding protein (CAB) and senescence-activated genes 12 and 13 (SAG12 and SAG13). mRNA transcript levels were assayed by Northern blot analysis at 0, 4, 8 days after the plants bolted. The CAB gene encodes the *Arabidopsis* chlorophyll binding protein which is involved in capturing light for photosynthesis. It is required for the green color of the leaf and is a good marker of chlorophyll turnover in the plant. CAB in wild-type plants shows transcript level reduction upon induction of senescence. No transcript level reduction was observed in aging leaves of *era*1-2 mutants. SAG12 and SAG13 are *Arabidopsis* genes cloned by differential expression during senescence (SAG stands for senescence activated gene). Transcription of both genes is induced during the onset of senescence in wild-type *Arabidopsis* plants. These genes were not induced under the same developmental conditions in the *era*1-2 mutants.

Example 9: Cloning of *Arabidopsis thaliana* FTA and Construction of Transformation Vector

The *Arabidopsis thaliana* FTA sequence was obtained by RT-PCR from total RNA isolated from leaf tissue using primers corresponding to SEQ ID NO:17 and SEQ ID NO:18. The resulting fragment was digested with *Bam*HI and *Sma*I and cloned into the plasmid pCR2.1 The Clonetech vector pBI121 was used as the backbone for the antisense construct. The GUS gene was removed by *Bam*HI and *Eco*1CRI digestion and replaced with the FTA insert that was cut from pCR2.1-FTA using *Sma*I and *Bam*HI and ligated into the vector SEQ ID NO: 10.

Table 1.

SEQ ID NO:17: 5'- AAAGGATCCTCAAATTGCTGCCACTGTAAT -3'

SEQ ID NO:18: 5'- AAACCCGGGATGAATTTCGACGAGAACGTG -3'

Example 10: Cloning of non-full length *Brassica napus* FTA and FTB nucleic acid sequences

RNA was isolated from leaf and root tissue using the Qiagen RNeasy kit. RT-PCR was performed by known techniques using the primers shown in Table 2. The FTA sequence was obtained using the primer pair SEQ ID NO:25 and SEQ ID NO:26. The FTB sequence was obtained using the primer pair SEQ ID NO:27 and SEQ ID NO:28.

Table 2.

SEQ ID NO:25: 5'-GGATCCATGGATTACTTCCGTGCGATTTACTTCTCC-3'

SEQ ID NO:26: 5'-AAAAAGCTTCCATGCCCAATAGTTAGCTCTTATTGGATC-3'

SEQ ID NO:27: 5'-AAAAAGCTTTGGCTTTGTTACTGGATTCTTCATTCAAT-3'

SEQ ID NO:28: 5'-AAATCTAGAAGCTTCATAATACCGATCCAAGACAATGTT-3'

PCR products were separated from the RT-PCR reaction mixture using the Qiagen PCR column spin kit and ligated into the cloning vector pBluescript KS +. The vector was digested with *Eco*RV and treated with *Taq* polymerase in the presence of dTTP to produce a 3' overhang for ligation with the PCR products. The ligation products were transformed into *E. coli* DH5α cells, positive colonies were selected and the resulting inserts sequenced.

Example 11: Cloning of non-full length FTA and FTB nucleic acid sequences from Glycine max and Zea maize

RNA was isolated from leaf and root tissue using the Qiagen RNeasy kit. RT-PCR was performed by known techniques using the primers shown in Table 3. The *Glycine max* FTA sequence was obtained using the primer pair SEQ ID NO:29 and SEQ ID NO:30. The *Glycine max* FTB sequence was obtained using the primer pair SEQ ID NO:31 and SEQ ID NO:32. The *Zea maize* FTB sequence was obtained using the primer pair SEQ ID NO:33 and SEQ ID NO:34

Table 3.

SEQ ID NO:29: 5'-AAAGGATCCATGGAATCTGGGTCTAGCGA-3'

SEQ ID NO:30: 5'-AAATCTAGAAGGAAGTCTGCTCTTGCGC-3'

SEQ ID NO:31: 5'-AAATCTAGAGCCACCATTCCTCGCAACG-3'

SEQ ID NO:32: 5'-AAAGAGCTCGTGGTGGAGAATCTGGGTGC-3'

SEQ ID NO:33: 5'-GGCGGATCCCGACCTACCGAGG-3'

SEQ ID NO:34: 5'-AAAGAGCTCGTGGATGGATTGGCTCCAGC-3'

PCR products were separated from the RT-PCR reaction mixture using the Qiagen PCR column spin kit and ligated into the cloning vector pBluescript KS +. The vector was digested with *Eco*RV and treated with *Taq* polymerase in the presence of dTTP to produce a 3' overhang for ligation with the PCR products. The ligation products were transformed into *E. coli* DH5α cells, positive colonies were selected and the resulting inserts sequenced.

Example 12: Sequence Analysis

Arabidopsis thaliana FTA

A disclosed nucleic acid of 999 nucleotides (also referred to as FT1) is shown in Table 4A. The primers used in the PCR are depicted in bold.

Table 4A. FT1 Nucleotide Sequence (SEQ ID NO:7).

Aaacccgggatgaatttcgacgagaccgtgccactgagccaacgattggagtggtcagacgtggt
cccattgactcaggacgatggtccgaatccagtggtgccaattgcctacaaggaagagttccgcg
agactatggattacttccgtgcgatttacttttccgacgagcgatctcctcgcgcactacgactc
acggaagaaaccctcctcttaaactccggcaactacacagtgtggcatttcaggcgcctagtact
cgaggcccttaatcacgacttgtttgaagaactcgagttcatcgaacgcattgctgaggataact
ctaagaactaccaactgtggcatcatcggcgatgggttgcagagaaactgggtcctgatgttgca
gggagagaacttgaatttacccgtagagtactttcacttgatgccaaacattatcatgcttggtc
acataggcagtggacactacgggcattaggaggatgggaagatgagctcgattactgtcacgagc
tccttgaagctgacgtctttaacaattccgcctggaatcagaggtattatgtcatcacccaatct
cctttgttgggaggcctagaagccatgagagaatctgaagtaagctacacaatcaaagccatttt
aaccaatcctgcaaacgagagctcatggcgatacctaaaagcgctttacaaagacgacaaagaat
cctggattagtgatccaagtgtttcctcagtctgtttgaatgttctatcccgcacagattgcttc
catggattcgctctgagcacccttttggatcttctatgtgatggactgagaccaaccaacgagca
taaagactcagtgagagctctagctaatgaagaaccagagactaacttggccaatttggtgtgta
ctattcttggtcgtgtagatcctataagagctaactattgggcatggaggaagagcaagatt**aca**
gtggcagcaatttgaggatccttt

A disclosed FT1 polypeptide (SEQ ID NO:11) encoded by SEQ ID NO:7 has 326 amino acid residues and is presented in Table 4B using the one-letter amino acid code.

Table 4B. Encoded FT1 protein sequence (SEQ ID NO:11).

MNFDETVPLSQRLEWSDVVPLTQDDGPNPVVPIAYKEEFRETMDYFRAIYFSDERSPRALRLTE
ETLLLNSGNYTVWHFRRLVLEALNHDLFEELEFIERIAEDNSKNYQLWHHRRWVAEKLGPDVAG
RELEFTRRVLSLDAKHYHAWSHRQWTLRALGGWEDELDYCHELLEADVFNNSAWNQRYYVITQS
PLLGGLEAMRESEVSYTIKAILTNPANESSWRYLKALYKDDKESWISDPSVSSVCLNVLSRTDC
FHGFALSTLLDLLCDGLRPTNEHKDSVRALANEEPETNLANLVCTILGRVDPIRANYWAWRKSK
ITVAAI

Due to the nature of the cloning strategy the sequence presented does not contain any 5' or 3' non-translated sequence. Using the sequences disclosed herein as hybridization probes, one is able to screen and isolate full length sequences from cDNA or genomic libraries or use the

rapid amplification of cDNA ends (RACE) technology or other such PCR techniques. The percent identity of the *Arabidopsis thaliana* nucleotide sequence and its encoded amino acid sequence to that of published sequences is shown in Figure 17.

The present invention also includes a nucleic acid sequence complimentary to the *Arabidopsis thaliana* farnesyl transferase alpha subunit of SEQ ID NO:7. The disclosed complimenary sequence is shown as SEQ ID NO:8. The nucleic acid sequence of SEQ ID NO:9 shows the nucleic acid sequence of SEQ ID NO:8 that has been prepared for ligation into an expression vector.

SEQ ID NO:8

aaaggatcctcaaattgctgccactgtaatcttgctcttcctccatgcccaatagttagctctt
ataggatctacacgaccaagaatagtacacaccaaattggccaagttagtctctggttcttcat
tagctagagctctcactgagtctttatgctcgttggttggtctcagtccatcacatagaagatc
caaaagggtgctcagagcgaatccatggaagcaatctgtgcgggatagaacattcaaacagact
gaggaaacacttggatcactaatccaggattctttgtcgtctttgtaaagcgcttttaggtatc
gccatgagctctcgtttgcaggattggttaaaatggctttgattgtgtagcttacttcagattc
tctcatggcttctaggcctcccaacaaaggagattgggtgatgacataatacctctgattccag
gcggaattgttaaagacgtcagcttcaaggagctcgtgacagtaatcgagctcatcttcccatc
ctcctaatgcccgtagtgtccactgcctatgtgaccaagcatgataatgtttggcatcaagtga
aagtactctacgggtaaattcaagttctctccctgcaacatcaggacccagtttctctgcaacc
catcgccgatgatgccacagttggtagttcttagagttatcctcagcaatgcgttcgatgaact
cgagttcttcaaacaagtcgtgattaagggcctcgagtactaggcgcctgaaatgccacactgt
gtagttgccggagtttaagaggagggtttcttccgtgagtcgtagtgcgcgaggagatcgctcg
tcggaaaagtaaatcgcacggaagtaatccatagtctcgcggaactcttccttgtaggcaattg
gcaccactggattcggaccatcgtcctgagtcaatgggaccacgtctgaccactccaatcgttg
gctcagtgg**cacggtctcgtcgaaattcatcccgggttt**

SEQ ID NO:9

gatcctcaaattgctgccactgtaatcttgctcttcctccatgcccaatagttagctcttatag
gatctacacgaccaagaatagtacacaccaaattggccaagttagtctctggttcttcattagc
tagagctctcactgagtctttatgctcgttggttggtctcagtccatcacatagaagatccaaa
agggtgctcagagcgaatccatggaagcaatctgtgcgggatagaacattcaaacagactgagg
aaacacttggatcactaatccaggattctttgtcgtctttgtaaagcgcttttaggtatcgcca
tgagctctcgtttgcaggattggttaaaatggctttgattgtgtagcttacttcagattctctc
atggcttctaggcctcccaacaaaggagattgggtgatgacataatacctctgattccaggcgg
aattgttaaagacgtcagcttcaaggagctcgtgacagtaatcgagctcatcttcccatcctcc
taatgcccgtagtgtccactgcctatgtgaccaagcatgataatgtttggcatcaagtgaaagt
actctacgggtaaattcaagttctctccctgcaacatcaggacccagtttctctgcaacccatc
gccgatgatgccacagttggtagttcttagagttatcctcagcaatgcgttcgatgaactcgag
ttcttcaaacaagtcgtgattaagggcctcgagtactaggcgcctgaaatgccacactgtgtag
ttgccggagtttaagaggagggtttcttccgtgagtcgtagtgcgcgaggagatcgctcgtcgg
aaaagtaaatcgcacggaagtaatccatagtctcgcggaactcttccttgtaggcaattggcac
cactggattcggaccatcgtcctgagtcaatgggaccacgtctgaccactccaatcgttggctc
agtgg***cacggtctcgtcgaaattcatccc***

Brassica napus FTA

A disclosed nucleic acid of 822 nucleotides (also referred to as FT2) is shown in Table 5A.

Table 5A. FT2 Nucleotide Sequence (SEQ ID NO:12).

ATGGATTACTTCCGTGCGATTTACTTCTCCGACGAGCGTTCTGCTCGCGCGCTGCGACTCACGGA
AGAAGCTCTCCGCTTAAACTCGGGCAACTACACCGTGTGGCACTTCGGGCGCTTAGTACTCGAGG
AGCTTAATAACGACTTGTATGAAGAGCTCAAGTTCATCGAAAGCATTGCTGAGGATAACTCTAAG
AACTACCAGTTGTGGCATCATCGACGATGGGTCGCAGAGAAACTGGGTCCTGATGTTGCAGGAAA
GGAACTTGAGTTTACTCGGAGGGTACTATCACTTGATGCCAAGCATTATCATGCTTGGTCACATA
GGCAGTGGGCGCTACAAGCATTAGGAGGATGGGAAAATGAGCTTAACTACTGCCACGAGCTCCTT
GAAGCTGACGTCTTTAACAACTCTGCATGGAATCAGAGGTATTACGTTATAACTAGATCACCTTC
GTTGGGAGGCCTAGAAGCCATGAGAGAATCTGAAGTAAGCTACACAGTCAAAGCCATTTTAGCAA
ATCCCGGGAACGAGAGCTCTTGGAGGTACCTGAAAGCCCTTTACAAAGACGACACAGAGTCTTGG
ATTAGTGATCCAAGTGTTTCCTCAGTCTGTTTGAAAGTTCTCTCACGCGCGGACTGCTTCCATGG
ATTCGCTCTGAGCACCCTTTTGGATCTTCTGTGCGATGGGTTGAGACCAACCAACGAGCATAGAG
ACTCGGTGAAAGCTCTAGCTAATGAAGAACCAGAGACTAACTTGGCCAATTTGGTGTGTACCATT
CTGTGTCGTGTTGATCCAATAAGAGCTAACTATTGGGCATGG

A disclosed FT2 polypeptide (SEQ ID NO:13) encoded by SEQ ID NO:12 has 274 amino acid residues and is presented in Table 5B using the one-letter amino acid code.

Table 5B. Encoded FT2 protein sequence (SEQ ID NO:13).

MDYFRAIYFSDERSARALRLTEEALRLNSGNYTVWHFGRLVLEELNNDLYEELK
FIESIAEDNSKNYQLWHHRRWVAEKLGPDVAGLEKEFTRRVLSLDAKHYHAWSH
RQWALQALGGWENELNYCHELLEADVFNNSAWNQRYYVITRSPSLGGLEAMRES
EVSYTVKAILANPGNESSWRYLKALYKDDTESWISDPSVSSVCLKVLSRADCFH
GFALSTLLDLLCDGLRPTNEHRDSVKALANEEPETNLANLVCTILCRVDPIRAN
YWAWKL

Due to the nature of the cloning strategy the sequence presented is not full length. Compared to the *Arabidopsis thaliana* sequence there are 42 amino acids missing from the amino terminus and 10 amino acids from the carboxy terminus. The percent identity of the *Brassica napus* nucleotide sequence and its encoded amino acid sequence to that of published sequences is shown in Figure 17.

Using the sequences disclosed herein as hybridization probes, one is able to screen and isolate full length sequences from cDNA or genomic libraries or use the rapid amplification of cDNA ends (RACE) technology or other such PCR techniques.

The present invention also includes a nucleic acid sequence complimentary to the *Brassica napsus* farnesyl transferase alpha subunit of SEQ ID NO:12. The disclosed complimenary sequence is shown as SEQ ID NO:35.

SEQ ID NO:35

CCATGCCCAATAGTTAGCTCTTATTGGATCAACACGACACAGAATGGTACACACCAAATTGGCC
AAGTTAGTCTCTGGTTCTTCATTAGCTAGAGCTTTCACCGAGTCTCTATGCTCGTTGGTTGGTC
TCAACCCATCGCACAGAAGATCCAAAAGGGTGCTCAGAGCGAATCCATGGAAGCAGTCCGCGCG
TGAGAGAACTTTCAAACAGACTGAGGAAACACTTGGATCACTAATCCAAGACTCTGTGTCGTCT
TTGTAAAGGGCTTTCAGGTACCTCCAAGAGCTCTCGTTCCCGGGATTTGCTAAAATGGCTTTGA
CTGTGTAGCTTACTTCAGATTCTCTCATGGCTTCTAGGCCTCCCAACGAAGGTGATCTAGTTAT
AACGTAATACCTCTGATTCCATGCAGAGTTGTTAAAGACGTCAGCTTCAAGGAGCTCGTGGCAG
TAGTTAAGCTCATTTTCCCATCCTCCTAATGCTTGTAGCGCCCACTGCCTATGTGACCAAGCAT
GATAATGCTTGGCATCAAGTGATAGTACCCTCCGAGTAAACTCAAGTTCCTTTCCTGCAACATC
AGGACCCAGTTTCTCTGCGACCCATCGTCGATGATGCCACAACTGGTAGTTCTTAGAGTTATCC
TCAGCAATGCTTTCGATGAACTTGAGCTCTTCATACAAGTCGTTATTAAGCTCCTCGAGTACTA
AGCGCCCGAAGTGCCACACGGTGTAGTTGCCCGAGTTTAAGCGGAGAGCTTCTTCCGTGAGTCG
CAGCGCGCGAGCAGAACGCTCGTCGGAGAAGTAAATCGCACGGAAGTAATCCAT

Brassica napus FTB

A disclosed nucleic acid of 1110 nucleotides (also referred to as FT3) is shown in Table 6A.

Table 6A. FT3 Nucleotide Sequence (SEQ ID NO:14).

TGGCTTTGTTACTGGATTCTTCATTCAATTGCTTTGCTTGGGGAGTCTGTGGATGATGACTTAGA
AAACAATGCAATCGATTTTCTTGGACGTTGCCAGGGTTCTGATGGTGGATATGGTGGTGGTCCTG
GCCAACTTCCACATCTTGCAACAAGTTATGCTGCAGTGAATACACTTGTTACTTTAGGAGGTGAG
AAAGCCTTCTCTTCAATTAACAGAGAACAAATGGCTTGTTTCTTAAGACGAATGAAGGATACAAA
TGGAGGTTTCAGGATGCATAATATGGGAGAAATAGATGTGCGAGCGTGCTACACTGCGATTTTGA
TTGCAAGCATCCTGAACATTGTGGATGATGAACTCACCCGCGGCTTAGGAGATTACATTTTGAGT
TGCCAAACTTATGAAGGTGGCATTGGAGGGGAACCTGGCTCCGAAGCTCATGGTGGGTACACGTA
CTGTGGGTTGGCTACTATGATTTTAATCAATGAAGTCGACCGCTTGAATTTGGATTCGTTAATGA
ATTGGGTTGTACATCGACAAGGAGTAGAAATGGGATTCCAAGGTAGGACGAACAAATTGGTCGAC
GGTTGCTACACGTTTTGGCAGGCAGCCCCCTGTGTTCTACTACAGCGATTTTTTTCATCCCAGGA
TATGGCACCTCATGGATCATCATCACATATGTCACAAGGGACAGATGAAGATCACGAGGAACATG
GTCATGATGAAGATGATCCTGAAGACAGTGATGAAGATGATTCTGATGAGGATAGCGATGAAGAT
TCAGGGAATGGTCACCAAGTTCATCATACGTCTACCTACATTGACAGGAGAATTCAACCTGTTTT
TGATAGCCTCGGCTTGCAAAGATATGTGCTCTTGTGCTCTCAGGTTGCTGATGGTGGATTCAGAG
ACAAGCTGAGGAAACCCCGTGACTTCTACCACACATGTTACTGCCTAAGCGGTCTTTCCGTGGCT
CAACACGCTTGGTCAAAAGACGAGGACACTCCTCCTTTGACTCGTGACATTTTGGGTGGCTACGC
AAACCACCTTGAACCTGTTCACCTCCTCCACAACATTGTCTTGGATCGGTATTATGAAGCTTCTA
GATTT

A disclosed FT3 polypeptide (SEQ ID NO:15) encoded by SEQ ID NO:13 has 370 amino acid residues and is presented in Table 6B using the one-letter amino acid code.

Table 6B. Encoded FT3 protein sequence (SEQ ID NO:15).

WLCYWILHSIALLGESVDDDLENNAIDFLGRCQGSDGGYGGGPGQLPHLATSYA
AVNTLVTLGGEKAFSSINREQMACFLRRMKDTNGGFRMHNMGEIDVRACYTAIL
IASILNIVDDELTRGLGDYILSCQTYEGGIGGEPGSEAHGGYTYCGLATMILIN
EVDRLNLDSLMNWVVHRQGVEMGFQGRTNKLVDGCYTFWQAAPCVLLQRFFSSQ
DMAPHGSSSHMSQGTDEDHEEHGHDEDDPEDSDEDDSDEDSDEDSGNGHQVHHT
STYIDRRIQPVFDSLGLQRYVLLCSQVADGGFRDKLRKPRDFYHTCYCLSGLSV
AQHAWSKDEDTPPLTRDILGGYANHLEPVHLLHNILVDRYYEASRF

Due to the nature of the cloning strategy the sequence presented is not full length. Compared to the *Arabidopsis thaliana* sequence there are 31 amino acids missing from the amino terminus and 5 amino acids from the carboxy terminus. The percent identity of the *Brassica napus* nucleotide sequence and its encoded amino acid sequence to that of published sequences is shown in Figure 18.

Using the sequences disclosed herein as hybridization probes, one is able to screen and isolate full length sequences from cDNA or genomic libraries or use the rapid amplification of cDNA ends (RACE) technology or other such PCR techniques. Sequence comparisons have been performed and percent identities are shown in Figure 17 and Figure 18.

The present invention also includes a nucleic acid sequence complimentary to the *Brassica napsus* farnesyl transferase beta subunit of SEQ ID NO:14. The disclosed complimenary sequence is shown as SEQ ID NO:36.

SEQ ID NO:36

AAATCTAGAAGCTTCATAATACCGATCCAAGACAATGTTGTGGAGGAGGTGAACAGGTTCAAGG
TGGTTTGCGTAGCCACCCAAAATGTCACGAGTCAAAGGAGGAGTGTCCTCGTCTTTTGACCAAG
CGTGTTGAGCCACGGAAAGACCGCTTAGGCAGTAACATGTGTGGTAGAAGTCACGGGGTTTCCT
CAGCTTGTCTCTGAATCCACCATCAGCAACCTGAGAGCACAAGAGCACATATCTTTGCAAGCCG
AGGCTATCAAAAACAGGTTGAATTCTCCTGTCAATGTAGGTAGACGTATGATGAACTTGGTGAC
CATTCCCTGAATCTTCATCGCTATCCTCATCAGAATCATCTTCATCACTGTCTTCAGGATCATC
TTCATCATGACCATGTTCCTCGTGATCTTCATCTGTCCCTTGTGACATATGTGATGATGATCCA
TGAGGTGCCATATCCTGGGATGAAAAAAATCGCTGTAGTAGAACACAGGGGGCTGCCTGCCAAA
ACGTGTAGCAACCGTCGACCAATTTGTTCGTCCTACCTTGGAATCCCATTTCTACTCCTTGTCG
ATGTACAACCCAATTCATTAACGAATCCAAATTCAAGCGGTCGACTTCATTGATTAAAATCATA
GTAGCCAACCCACAGTACGTGTACCCACCATGAGCTTCGGAGCCAGGTTCCCCTCCAATGCCAC
CTTCATAAGTTTGGCAACTCAAAATGTAATCTCCTAAGCCGCGGGTGAGTTCATCATCCACAAT
GTTCAGGATGCTTGCAATCAAAATCGCAGTGTAGCACGCTCGCACATCTATTTCTCCCATATTA
TGCATCCTGAAACCTCCATTTGTATCCTTCATTCGTCTTAAGAAACAAGCCATTTGTTCTCTGT
TAATTGAAGAGAAGGCTTTCTCACCTCCTAAAGTAACAAGTGTATTCACTGCAGCATAACTTGT
TGCAAGATGTGGAAGTTGGCCAGGACCACCACCATATCCACCATCAGAACCCTGGCAACGTCCA
AGAAAATCGATTGCATTGTTTTCTAAGTCATCATCCACAGACTCCCCAAGCAAAGCAATTGAAT
GAAGAATCCAGTAACAAAGCCA

Glycine max FTA

A disclosed nucleic acid of 1041 nucleotides (also referred to as FT4) is shown in Table 7A.

Table 7A. FT4 Nucleotide Sequence (SEQ ID NO:37).

```
ATGGAATCTGGGTCTAGCGAAGGAGAAGAGGTGCAGCAACGCGTGCCGTTGAGGGAGAGAGTGGA
GTGGTCAGATGTTACTCCGGTTCCTCAAAACGACGGCCCTAACCCTGTCGTTCCGATCCAGTACA
CTGAAGAGTTTTCCGAAGTTATGGATTACTTTCGCGCCGTTTACCTCACCGATGAACGCTCCCCT
CGCGCCCTCGCTCTCACAGCCGAAGCCGTTCAATTCAACTCCGGCAACTACACTGTGTGGCATTT
CCGACGGTTGTTACTTGAGTCGCTAAAAGTCGACTTGAACGATGAACTGGAGTTTGTGGAGCGTA
TGGCCGCTGGAAATTCTAAAAATTATCAGATGTGnATGTTCTGTAGGCATCCTAGACGATGGGTT
GCCGAGAAGTTAGGTCCTGAAGCTAGAAACAATGAGCTCGAGTTCACCAAAAAGATACTGTCCGT
TGATGCCAAACATTATCATGCATGGTCTCATAGACAGTGGGCTCTTCAAACACTAGGAGGATGGG
AAGATGAACTTAATTATTGCACAGAACTACTTAAAGAAGACATTTTTAACAATTCTGCTTGGAAT
CAGAGATATTTTGTCATAACAAGGTCTCCTTTCTTGGGGGGCCTAAAAGCTATGAGAGAGTCTGA
AGTGCTTTACACCATCGAAGCCATTATAGCCTACCCTGAAAATGAAAGCTCGTGGAGATATCTAC
GAGGACTTTATAAAGGTGAAACTACTTCATGGGTAAATGATCCTCAAGTTTCTTCAGTATGCTTA
AAGATTTTGAGAACTAAGAGCAACTACGTGTTTGCTCTTAGCACTATTTTAGATCTTATATGCTT
TGGTTATCAACCAAATGAAGACATTAGAGATGCCATTGACGCCTTAAAGACCGCAGATATGGATA
AACAAGATTTAGATGATGATGAGAAAGGGGAACAACAAAATTTAAATATAGCACGAAATATTTGT
TCTATCCTAAAACAAGTTGATCCAATTAGAACCAACTATTGGATTTGGCGCAAGAGCAGACTTCC
T
```

A disclosed FT4 polypeptide (SEQ ID NO:39) encoded by SEQ ID NO:37 has 347 amino acid residues and is presented in Table 7B using the one-letter amino acid code.

Table 7B. Encoded FT4 protein sequence (SEQ ID NO:39).

```
MESGSSEGEEVQQRVPLRERVEWSDVTPVPQNDGPNPVVPIQYTEEFSEVMDYF
RAVYLTDERSPRALALTAEAVQFNSGNYTVWHFRRLLLESLKVDLNDELEFVER
MAAGNSKNYQMXMFCRHPRRWVAEKLGPEARNNELEFTKKILSVDAKHYHAWSH
RQWALQTLGGWEDELNYCTELLKEDIFNNSAWNQRYFVITRSPFLGGLKAMRES
EVLYTIEAIIAYPENESSWRYLRGLYKGETTSWVNDPQVSSVCLKILRTKSNYV
FALSTILDLICFGYQPNEDIRDAIDALKTADMDKQDLDDDEKGEQQNLNIARNI
CSILKQVDPIRTNYWIWRKSRLP
```

Due to the nature of the cloning strategy the sequence presented is not full length. The percent identity of the *Glycine max* nucleotide sequence and its encoded amino acid sequence to that of other sequences is shown in Figure 17.

Using the sequences disclosed herein as hybridization probes, one is able to screen and isolate full length sequences from cDNA or genomic libraries or use the rapid amplification of cDNA ends (RACE) technology or other such PCR techniques.

The present invention also includes a nucleic acid sequence complimentary to the *Glycine max* alpha subunit of SEQ ID NO:37. The disclosed complimenary sequence is shown as SEQ ID NO:38.

SEQ ID NO:38

```
AGGAAGTCTGCTCTTGCGCCAAATCCAATAGTTGGTTCTAATTGGATCAACTTGTTTTAGGATA
GAACAAATATTTCGTGCTATATTTAAATTTTGTTGTTCCCCTTTCTCATCATCATCTAAATCTT
GTTTATCCATATCTGCGGTCTTTAAGGCGTCAATGGCATCTCTAATGTCTTCATTTGGTTGATA
```

ACCAAAGCATATAAGATCTAAAATAGTGCTAAGAGCAAACACGTAGTTGCTCTTAGTTCTCAAA
ATCTTTAAGCATACTGAAGAAACTTGAGGATCATTTACCCATGAAGTAGTTTCACCTTTATAAA
GTCCTCGTAGATATCTCCACGAGCTTTCATTTTCAGGGTAGGCTATAATGGCTTCGATGGTGTA
AAGCACTTCAGACTCTCTCATAGCTTTTAGGCCCCCCAAGAAAGGAGACCTTGTTATGACAAAA
TATCTCTGATTCCAAGCAGAATTGTTAAAAATGTCTTCTTTAAGTAGTTCTGTGCAATAATTAA
GTTCATCTTCCCATCCTCCTAGTGTTTGAAGAGCCCACTGTCTATGAGACCATGCATGATAATG
TTTGGCATCAACGGACAGTATCTTTTTGGTGAACTCGAGCTCATTGTTTCTAGCTTCAGGACCT
AACTTCTCGGCAACCCATCGTCTAGGATGCCTACAGAACATNCACATCTGATAATTTTTAGAAT
TTCCAGCGGCCATACGCTCCACAAACTCCAGTTCATCGTTCAAGTCGACTTTTAGCGACTCAAG
TAACAACCGTCGGAAATGCCACACAGTGTAGTTGCCGGAGTTGAATTGAACGGCTTCGGCTGTG
AGAGCGAGGGCGCGAGGGGAGCGTTCATCGGTGAGGTAAACGGCGCGAAAGTAATCCATAACTT
CGGAAAACTCTTCAGTGTACTGGATCGGAACGACAGGGTTAGGGCCGTCGTTTTGAGGAACCGG
AGTAACATCTGACCACTCCACTCTCTCCCTCAACGGCACGCGTTGCTGCACCTCTTCTCCTTCG
CTAGACCCAGATTCCAT

Glycine max FTB

A disclosed nucleic acid of 1035 nucleotides (also referred to as FT5) is shown in Table 8A.

Table 8A. FT5 Nucleotide Sequence (SEQ ID NO:40).

GCCACCATTCCTCGCAACGCCCAAACCCTCATGTTGGAGCTTCAACGCGATAATCACATGCAGTA
TGTCTCCAAAGGCCTTCGCCATCTCAGTTCCGCATTTTCCGTTTTGGACGCTAATCGACCCTGGC
TCTGCTACTGGATCTTCCACTCCATTGCTTTGTTGGGAGAATCCGTCGATGATGAACTCGAAGAT
AACGCTATCGATTTTCTTAACCGTTGCCAGGATCCGAATGGTGGATATGCCGGGGGACCAGGCCA
GATGCCTCATATTGCCACAACTTATGCTGCTGTTAATTCACTTATTACTTTGGGTGGTGAGAAAT
CCCTGGCATCAATTAATAGAGATAAACTGTATGGGTTTCTGCGGCGGATGAAGCAACCAAATGGT
GGATTCAGGATGCATGATGAAGGTGAAATTGATGTTCGAGCTTGCTACACTGCCATTTCTGTTGC
AAGTGTTTTGAACATTTTGGATGATGAGCTGATCCAGAATGTTGGAGACTACATTATAAGCTGTC
AAACATATGAGGGTGGCATTGCTGGTGAGCCTGGTTCTGAGGCTCATGGTGGGTACACCTTTTGT
GGATTAGCTACAATGATTCTGATTGGTGAGGTTAATCACTTGGATCTGCCTCGATTAGTTGACTG
GGTGGTATTCCGACAAGGTAAGGAATGTGGATTCCAGGGGAGAACAAATAAACTGGTGGATGGAT
GCTATTCCTTTTGGCAGGGAGGTGCTGTTGCTCTATTGCAAAGATTATCTTCTATTATCAACAAA
CAGATGGAAGAGACATCACAGATTTTTGCGGTATCTTATGTATCTGAAGCAAAAGAAAGTTTGGA
TGGAACCTCTAGTCATGCAACATGCCGTGGTGAGCATGAAGGCACCAGTGAATCCAGTTCATCTG
ATTTTAAAAATATTGCCTATAAATTTATTAATGAGTGGAGAGCACAAGAACCACTTTTTCACAGT
ATTGCTTTACAGCAATATATTCTCTTATGTGCACAGGAGCAAGAGGGTGGACTGAGAGACAAACC
GGGTAAACGTAGAGATCATTATCACACATGTTACTGTTTAAGTGGACTCTCATTGTGCCAGTATA
GTTGGTCAAAGCACCCAGATTCTCCACCAC

A disclosed FT5 polypeptide (SEQ ID NO:42) encoded by SEQ ID NO:40 has 378 amino acid residues and is presented in Table 8B using the one-letter amino acid code.

Table 8B. Encoded FT5 protein sequence (SEQ ID NO:42).

ATIPRNAQTLMLELQRDNHMQYVSKGLRHLSSAFSVLDANRPWLCYWIFHSIAL
LGESVDDELEDNAIDFLNRCQDPNGGYAGGPGQMPHIATTYAAVNSLITLGGEK
SLASINRDKLYGFLRRMKQPNGGFRMHDEGEIDVRACYTAISVASVLNILDDEL
IQNVGDYIISCQTYEGGIAGEPGSEAHGGYTFCGLATMILIGEVNHLDLPRLVD
WVVFRQGKECGFQGRTNKLVDGCYSFWQGGAVALLQRLSSIINKQMEETSQIFA
VSYVSEAKESLDGTSSHATCRGEHEGTSESSSSDFKNIAYKFINEWRAQEPLFH
SIALQQYILLCAQEQEGGLRDKPGKRRDHYHTCYCLSGLSLCQYSWSKHPDSPP

Due to the nature of the cloning strategy the sequence presented is not full length. The percent identity of the *Glycine max* nucleotide sequence and its encoded amino acid sequence to that of other sequences is shown in Figure 17.

Using the sequences disclosed herein as hybridization probes, one is able to screen and isolate full length sequences from cDNA or genomic libraries or use the rapid amplification of cDNA ends (RACE) technology or other such PCR techniques.

The present invention also includes a nucleic acid sequence complimentary to the *Glycine max* beta subunit of SEQ ID NO:40. The disclosed complimenary sequence is shown as SEQ ID NO:41.

SEQ ID NO:41

GTGGTGGAGAATCTGGGTGCTTTGACCAACTATACTGGCACAATGAGAGTCCACTTAAACAGTA
ACATGTGTGATAATGATCTCTACGTTTACCCGGTTTGTCTCTCAGTCCACCCTCTTGCTCCTGT
GCACATAAGAGAATATATTGCTGTAAAGCAATACTGTGAAAAAGTGGTTCTTGTGCTCTCCACT
CATTAATAAATTTATAGGCAATATTTTTAAAATCAGATGAACTGGATTCACTGGTGCCTTCATG
CTCACCACGGCATGTTGCATGACTAGAGGTTCCATCCAAACTTTCTTTTGCTTCAGATACATAA
GATACCGCAAAAATCTGTGATGTCTCTTCCATCTGTTTGTTGATAATAGAAGATAATCTTTGCA
ATAGAGCAACAGCACCTCCCTGCCAAAAGGAATAGCATCCATCCACCAGTTTATTTGTTCTCCC
CTGGAATCCACATTCCTTACCTTGTCGGAATACCACCCAGTCAACTAATCGAGGCAGATCCAAG
TGATTAACCTCACCAATCAGAATCATTGTAGCTAATCCACAAAAGGTGTACCCACCATGAGCCT
CAGAACCAGGCTCACCAGCAATGCCACCCTCATATGTTTGACAGCTTATAATGTAGTCTCCAAC
ATTCTGGATCAGCTCATCATCCAAAATGTTCAAAACACTTGCAACAGAAATGGCAGTGTAGCAA
GCTCGAACATCAATTTCACCTTCATCATGCATCCTGAATCCACCATTTGGTTGCTTCATCCGCC
GCAGAAACCCATACAGTTTATCTCTATTAATTGATGCCAGGGATTTCTCACCACCCAAAGTAAT
AAGTGAATTAACAGCAGCATAAGTTGTGGCAATATGAGGCATCTGGCCTGGTCCCCCGGCATAT
CCACCATTCGGATCCTGGCAACGGTTAAGAAAATCGATAGCGTTATCTTCGAGTTCATCATCGA
CGGATTCTCCCAACAAAGCAATGGAGTGGAAGATCCAGTAGCAGAGCCAGGGTCGATTAGCGTC
CAAAACGGAAAATGCGGAACTGAGATGGCGAAGGCCTTTGGAGACATACTGCATGTGATTATCG
CGTTGAAGCTCCAACATGAGGGTTTGGGCGTTGCGAGGAATGGTGGC

Zea maize FTB

A disclosed nucleic acid of 1235 nucleotides (also referred to as FT6) is shown in Table 9A.

Table 9A. FT6 Nucleotide Sequence (SEQ ID NO:43).

```
GGCGGATCCCGACCTACCGAGGCTCACGGTGACGCAGGTGGAGCAGATGAAGGTGGAGGCCAGGG
TTGGCGACATCTACCGCTCCCTCTTCGGGGCCGCGCCCAACACGAAATCCATCATGCTAGAGCTG
TGGCGTGATCAGCATATCGAGTATCTGACGCCTGGGCTGAGGCATATGGGACCAGCCTTTCATGT
TCTAGATGCCAATCGCCCTTGGCTATGCTACTGGATGGTTCATCCACTTGCTTTGCTGGATGAAG
CACTTGATGATGATCTTGAGAATGATATCATAGACTTCTTAGCTCGATGTCAGGATAAAGATGGT
GGATATAGTGGTGGACCTGGACAGTTGCCTCACCTAGCTACGACTTATGCTGCTGTAAATACACT
TGTGACAATAGGGAGCGAAAGAGCATTGTCATCAATCAATAGGGGCAACCTGTACAATTTTATGC
TGCAGATGAAAGATGTATCAGGTGCTTTCAGAATGCATGATGGTGGCGAAATTGATGTCCGTGCT
TCCTACACCGCTATATCGGTTGCCAGCCTTGTGAATATTCTTGATTTTAAACTGGCAAAAGGTGT
AGGCGACTACATAGCAAGATGTCAAACTTATGAAGGTGGTATTGCTGGGGAGCCTTATGCTGAAG
CACATGGTGGGTATACATTCTGTGGATTGGCTGCTTTGATCCTGCTTAATGAGGCAGAGAAAGTT
GACTTGCCTAGTTTGATTGGCTGGGTGGCTTTTCGTCAAGGAGTGGAATGCGGATTTCAAGGACG
AACTAATAAATTGGTTGATGGTTGCTACTCCTTTTGGCAGGGAGCTGCCATTGCTTTCACACAAA
AGTTAATTACGATTGTTGATAAGCAATTGAGGTCCTCGTATTCCTGCAAAAGGCCATCAGGAGAG
GATGCCTGCAGCACCAGTTCATATGGGTGCACCGCGAATAAGTCTTCCTCTGCTGTGGACTATGC
GAAGTTTGGATTTGATTTTATACAACAGAGCAACCAAATTGGCCCACTCTTCCATAACATTGCCC
TGCAACAATACATCCTACTTTGTTCTCAGGTACTAGAGGGAGGCTTGAGGGATAAGCCTGGAAAG
AACAGAGATCACTATCATTCATGCTACTGCCTCAGTGGCCTCGCAGTTAGCCAGTACAGTGCCAT
GACTGATACTGGTTCGTGCCCATTACCTCAGCATGTGCTTGGACCGTACTCTAATTTGCTGGAGC
CAATCCATCC
```

A disclosed FT6 polypeptide (SEQ ID NO:45) encoded by SEQ ID NO:43 has 414 amino acid residues and is presented in Table 9B using the one-letter amino acid code.

Table 9B. Encoded FT6 protein sequence (SEQ ID NO:45).

```
ADPDLPRLTVTQVEQMKVEARVGDIYRSLFGAAPNTKSIMLELWRDQHIEYLTP
GLRHMGPAFHVLDANRPWLCYWMVHPLALLDEALDDDLENDIIDFLARCQDKDG
GYSGGPGQLPHLATTYAAVNTLVTIGSERALSSINRGNLYNFMLQMKDVSGAFR
MHDGGEIDVRASYTAISVASLVNILDFKLAKGVGDYIARCQTYEGGIAGEPYAE
AHGGYTFCGLAALILLNEAEKVDLPSLIGWVAFRQGVECGFQGRTNKLVDGCYS
FWQGAAIAFTQKLITIVDKQLRSSYSCKRPSGEDACSTSSYGCTANKSSSAVDY
AKFGFDFIQQSNQIGPLFHNIALQQYILLCSQVLEGGLRDKPGKNRDHYHSCYC
LSGLAVSQYSAMTDTGSCPLPQHVLGPYSNLLEPIH
```

Due to the nature of the cloning strategy the sequence presented is not full length. The percent identity of the *Glycine max* nucleotide sequence and its encoded amino acid sequence to that of other sequences is shown in Figure 17.

Using the sequences disclosed herein as hybridization probes, one is able to screen and isolate full length sequences from cDNA or genomic libraries or use the rapid amplification of cDNA ends (RACE) technology or other such PCR techniques.

The present invention also includes a nucleic acid sequence complimentary to the *Zea maize* beta subunit of SEQ ID NO:43. The disclosed complimenary sequence is shown as SEQ ID NO:44.

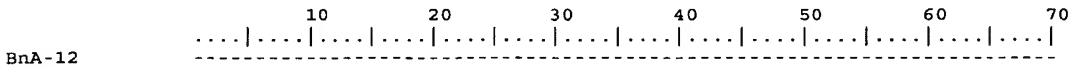
SEQ ID NO:44

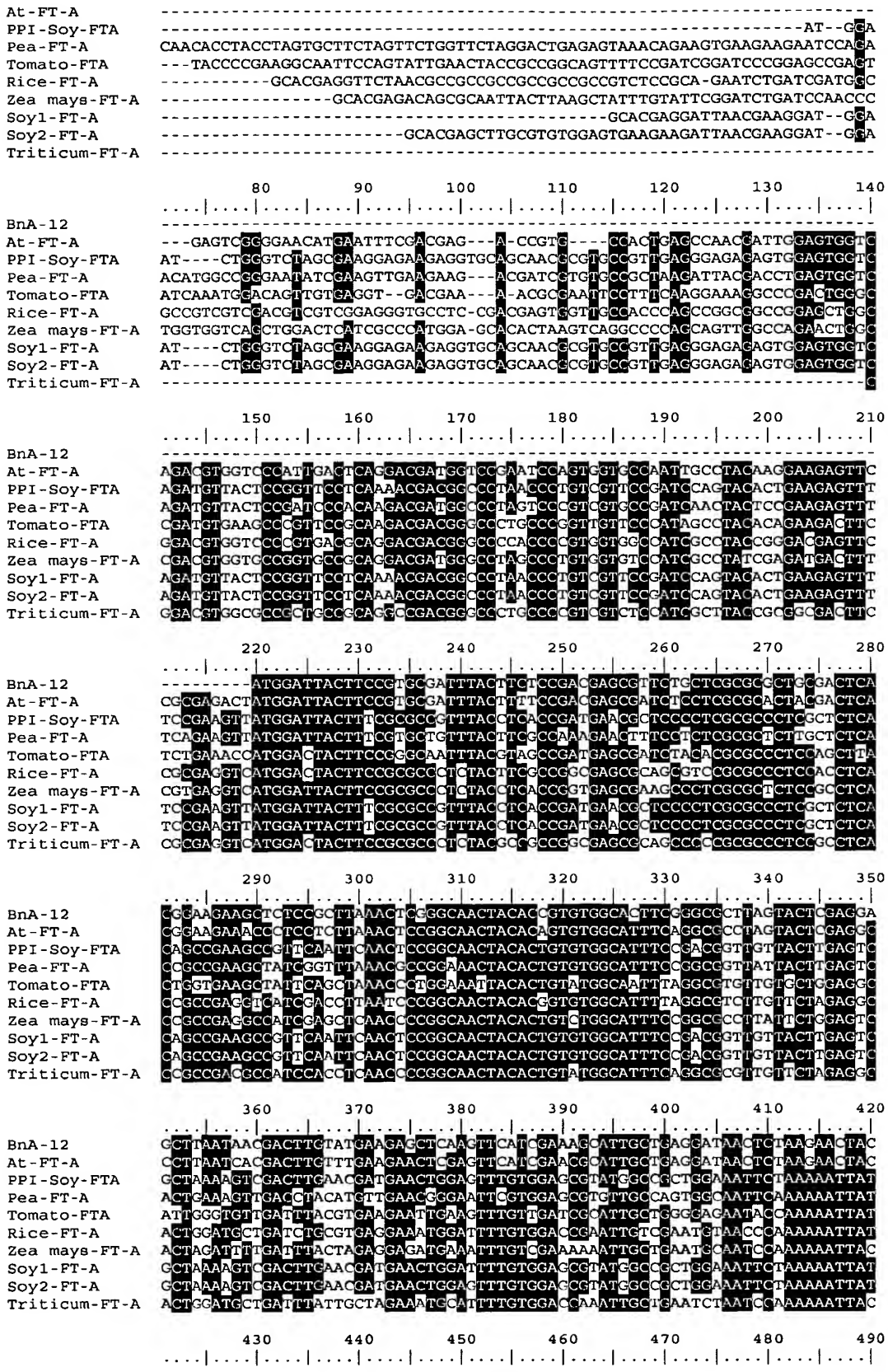
GGATGGATTGGCTCCAGCAAATTAGAGTACGGTCCAAGCACATGCTGAGGTAATGGGCACGAAC
CAGTATCAGTCATGGCACTGTACTGGCTAACTGCGAGGCCACTGAGGCAGTAGCATGAATGATA
GTGATCTCTGTTCTTTCCAGGCTTATCCCTCAAGCCTCCCTCTAGTACCTGAGAACAAAGTAGG
ATGTATTGTTGCAGGGCAATGTTATGGAAGAGTGGGCCAATTTGGTTGCTCTGTTGTATAAAAT
CAAATCCAAACTTCGCATAGTCCACAGCAGAGGAAGACTTATTCGCGGTGCACCCATATGAACT
GGTGCTGCAGGCATCCTCTCCTGATGGCCTTTTGCAGGAATACGAGGACCTCAATTGCTTATCA
ACAATCGTAATTAACTTTTGTGTGAAAGCAATGGCAGCTCCCTGCCAAAAGGAGTAGCAACCAT
CAACCAATTTATTAGTTCGTCCTTGAAATCCGCATTCCACTCCTTGACGAAAAGCCACCCAGCC
AATCAAACTAGGCAAGTCAACTTTCTCTGCCTCATTAAGCAGGATCAAAGCAGCCAATCCACAG
AATGTATACCCACCATGTGCTTCAGCATAAGGCTCCCCAGCAATACCACCTTCATAAGTTTGAC
ATCTTGCTATGTAGTCGCCTACACCTTTTGCCAGTTTAAAATCAAGAATATTCACAAGGCTGGC
AACCGATATAGCGGTGTAGGAAGCACGGACATCAATTTCGCCACCATCATGCATTCTGAAAGCA
CCTGATACATCTTTCATCTGCAGCATAAAATTGTACAGGTTGCCCCTATTGATTGATGACAATG
CTCTTTCGCTCCCTATTGTCACAAGTGTATTTACAGCAGCATAAGTCGTAGCTAGGTGAGGCAA
CTGTCCAGGTCCACCACTATATCCACCATCTTTATCCTGACATCGAGCTAAGAAGTCTATGATA
TCATTCTCAAGATCATCATCAAGTGCTTCATCCAGCAAAGCAAGTGGATGAACCATCCAGTAGC
ATAGCCAAGGGCGATTGGCATCTAGAACATGAAAGGCTGGTCCCATATGCCTCAGCCCAGGCGT
CAGATACTCGATATGCTGATCACGCCACAGCTCTAGCATGATGGATTTCGTGTTGGGCGCGGCC
CCGAAGAGGGAGCGGTAGATGTCGCCAACCCTGGCCTCCACCTTCATCTGCTCCACCTGCGTCA
CCGTGAGCCTCGGTAGGTCGGGATCCGCC

The FTA and FTB nucleic acids and amino acids disclosed above have homology to other members of the FT protein family (GenBank ID NOs: U63298, U83707, and U73203; WO 00/14207; Cutler et al., Science 273(5279):1239-41, 1996; Ziegelhoffer et al., Proc Natl Acad Sci U S A. 97(13):7633-8, 2000). The homology between these and other sequences is shown graphically in the ClustalW analysis shown in Tables 10A-10D. In the ClustalW alignment, the black outlined amino acid residues indicate regions of conserved sequence (*i.e.*, regions that may be required to preserve structural or functional properties), whereas non-highlighted amino acid residues are less conserved and can potentially be altered to a much broader extent without altering protein structure or function.

Table 10A. ClustalW Nucleic Acid Analysis of FT Alpha Subunits

1) BNA-12; FT2 (SEQ ID NO:12)
2) At-FT-A; FT1 (SEQ ID NO:7)
3) PPI-Soy-FTA; FT4 (SEQ ID NO:37)
4) Pea-FT-A (SEQ ID NO:65)
5) Tomato-FTA (SEQ ID NO:66)
6) Rice-FT-A (SEQ ID NO:67)
7) Zea mays-FT-A (SEQ ID NO:68)
8) Soy1-FT-A (SEQ ID NO:69)
9) Soy2-FT-A (SEQ ID NO:70)
10) Triticum-FT-A (SEQ ID NO:71)

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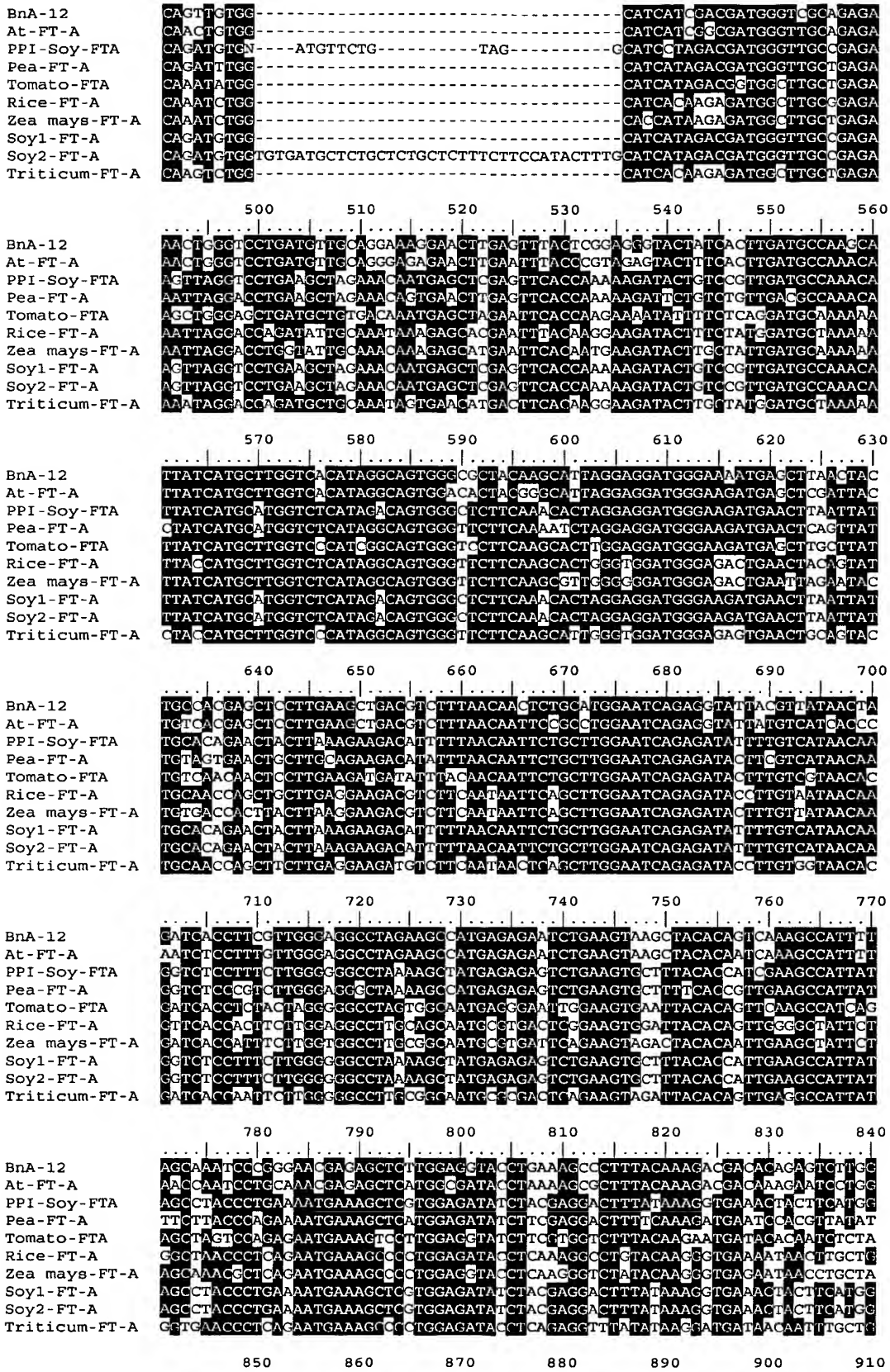
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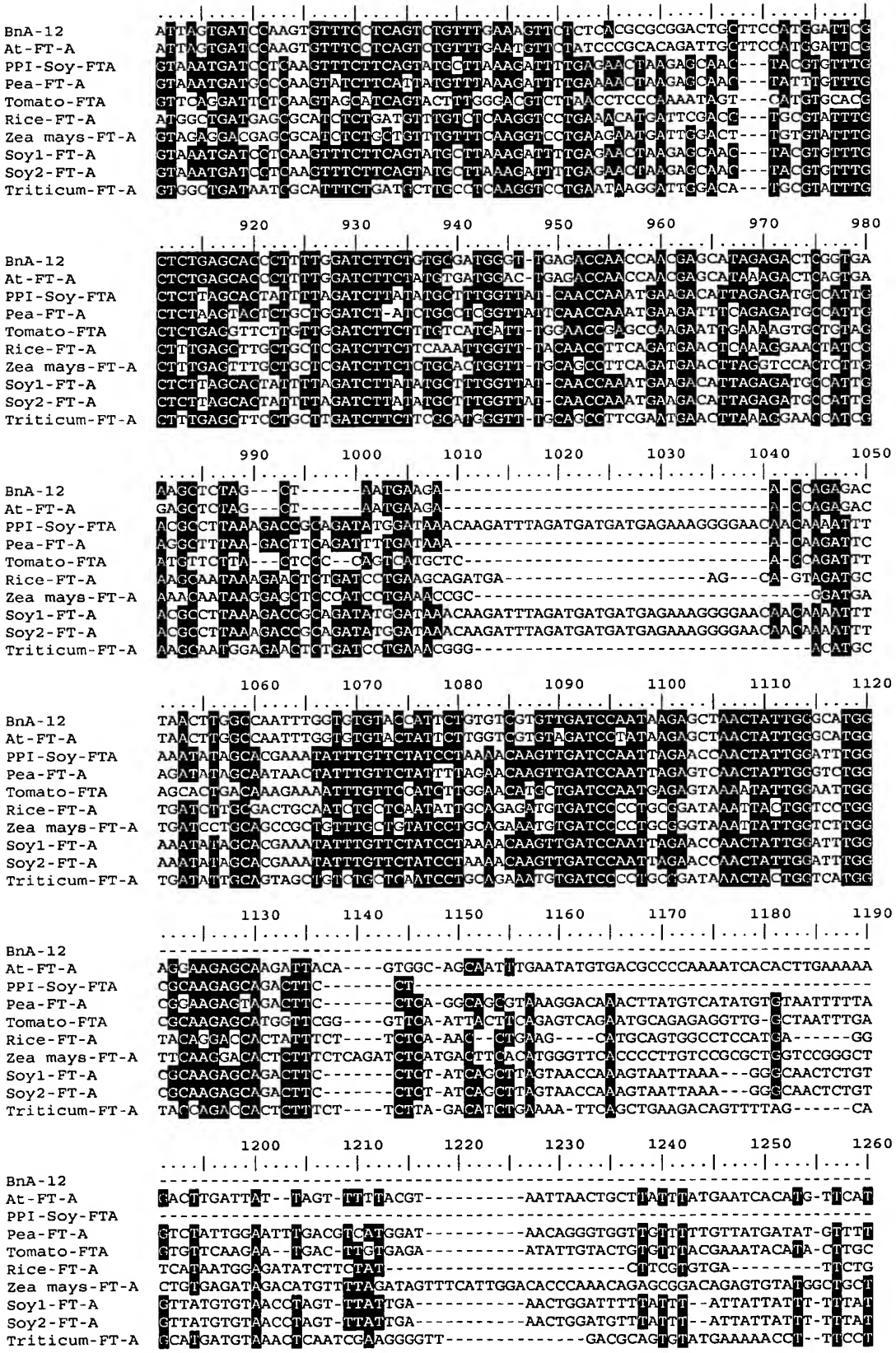
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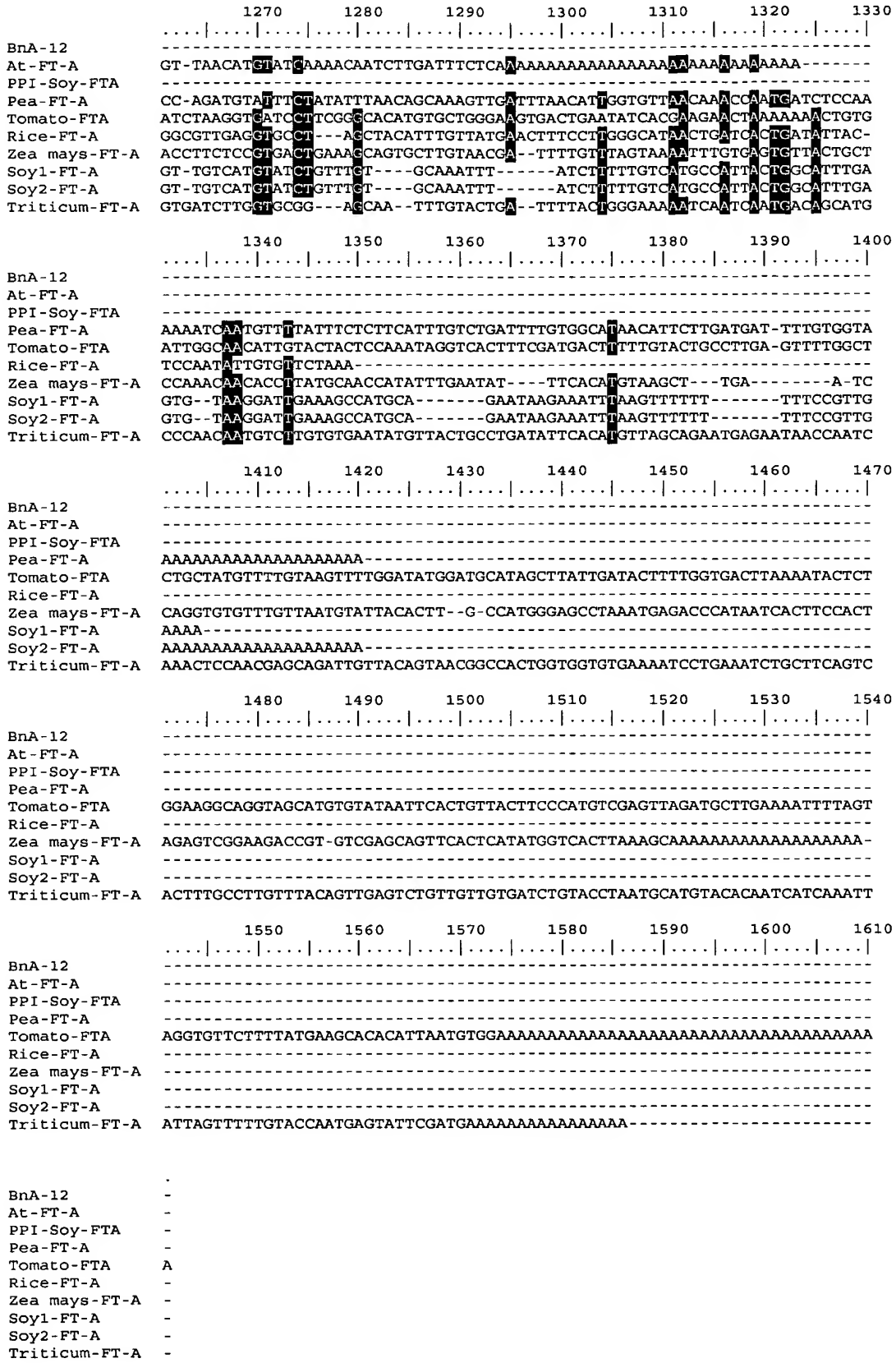
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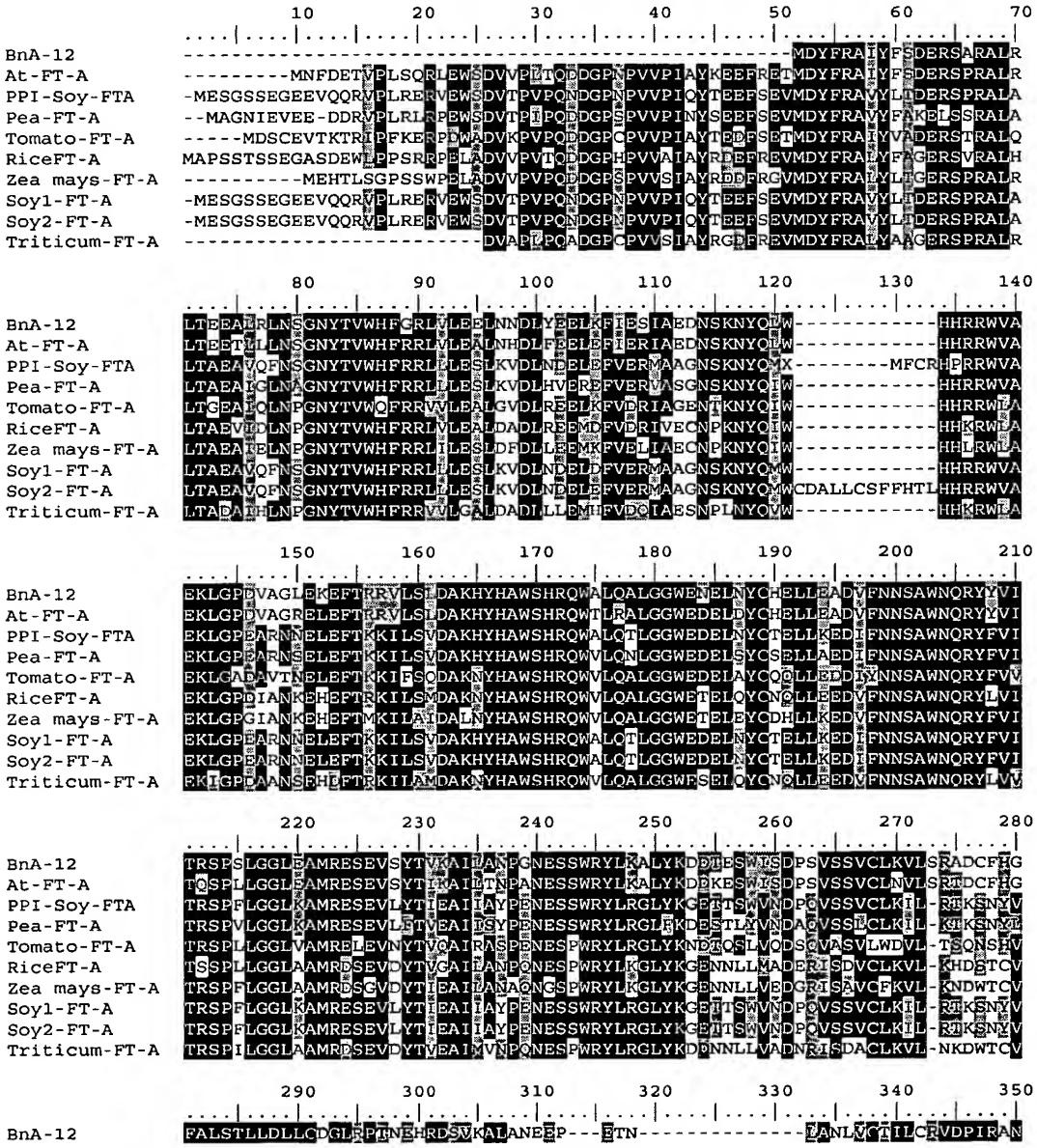
1410 1420 1430 1440 1450 1460 1470
1480 1490 1500 1510 1520 1530 1540
1550 1560 1570 1580 1590 1600 1610

Table 10B. ClustalW Amino Acid Analysis of FT Alpha Subunits

1) BNA-12; FT2 (SEQ ID NO:13)
2) At-FT-A; FT1 (SEQ ID NO:11)
3) PPI-Soy-FTA; FT4 (SEQ ID NO:39)
4) Pea-FT-A (SEQ ID NO:72)
5) Tomato-FTA (SEQ ID NO:73)
6) Rice-FT-A (SEQ ID NO:74)
7) Zea mays-FT-A (SEQ ID NO:75)
8) Soy1-FT-A (SEQ ID NO:76)
9) Soy2-FT-A (SEQ ID NO:77)
10) Triticum-FT-A (SEQ ID NO:78)



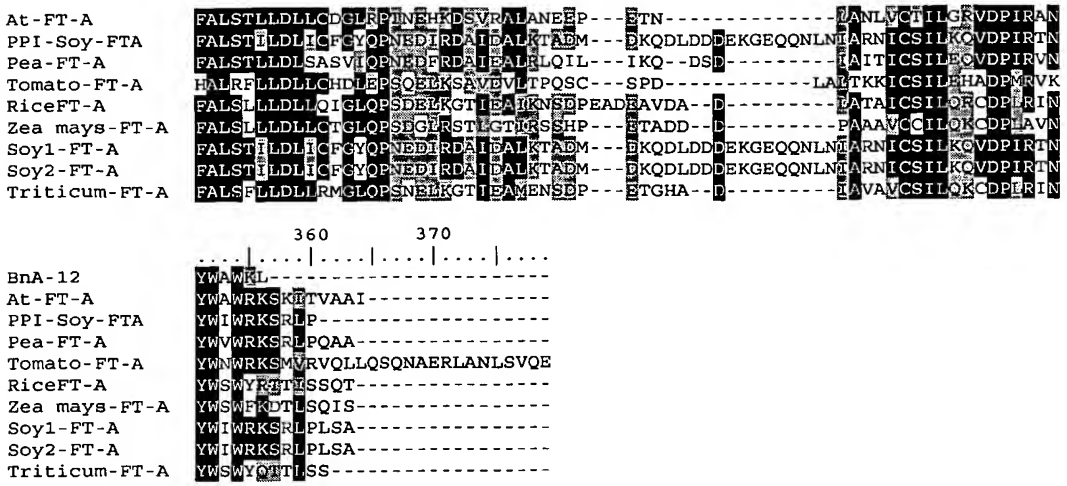
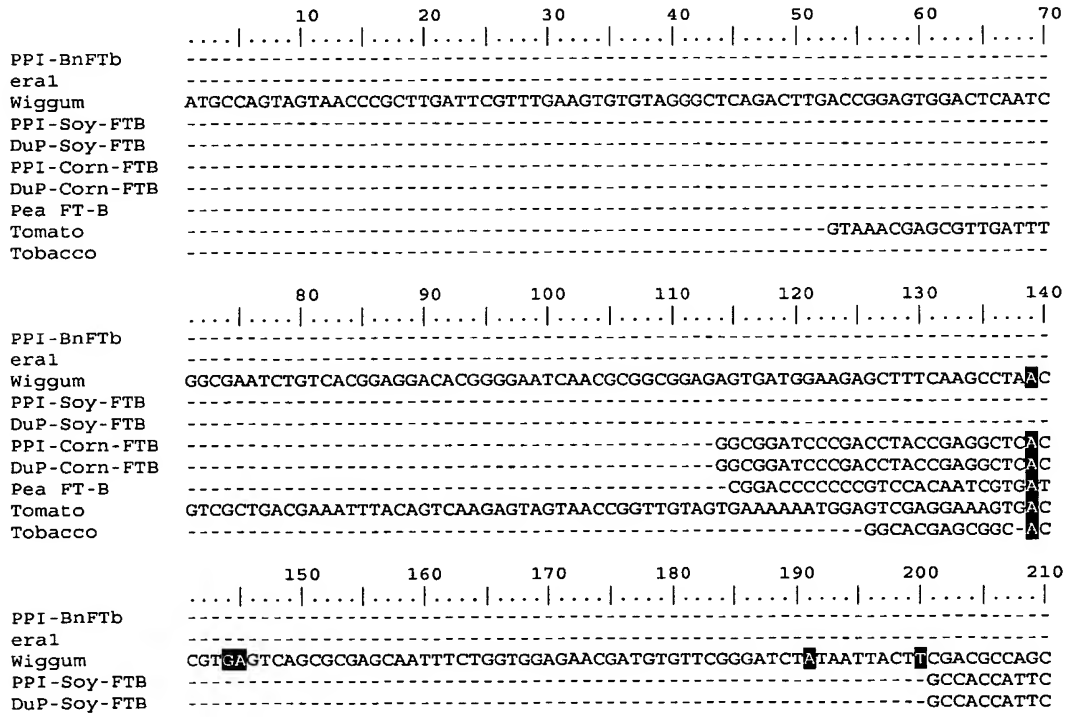


Table 10C. ClustalW Nucleic Acid Analysis of FT Beta Subunits

1) PPI-BnFTb; FT3 (SEQ ID NO:14)
2) era1 (SEQ ID NO:1)
3) Wiggum (SEQ ID NO:80)
4) PPI-Soy-FTB; FT5 (SEQ ID NO:40)
5) DuP-Soy-FTB (SEQ ID NO:81)
6) PPI-Corn-FTB; FT6 (SEQ ID NO:43)
7) DuP-Corn-FTB (SEQ ID NO:82)
8) Pea-FT-B (SEQ ID NO:83)
9) Tomato (SEQ ID NO:84)
10) Tobacco (SEQ ID NO:85)



```
PPI-Corn-FTB   GGTGACGCAGGTGGAGCAGATGAAGGTGGAGGCCAGGGTTGGCGACATCTACCGCTCCCTCTTCGGGGCC
DuP-Corn-FTB   GGTGACGCAGGTGGAGCAGATGAAGGTGGAGGCCAGGGTTGGCGACATCTACCGCTCCCTCTTCGGGGCC
Pea FT-B       GATGACGTCTCCGCGAGCATTTCAACAACCAGTTACTCAAACCACCGCGGAGTAACACATGGAAGCTTCA
Tomato         GAAGACGCTGGAAGATCAATGGGTGGTGGAGCGTCGAGTCCGAGAGATATACGATTATTTCTACAGCATT
Tobacco        GAGGACACTGGAAGATCAATGGATGGTGGAGCGTCAAGTTCGGGAGATATACAATTTTTTCTACAGCATT

                        220       230       240       250       260       270       280
               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
PPI-BnFTb      ----------------------------------------------------------------------
era1           ------------------------ATGGAGATTCAGCGAGATAAGCAATT-GGATTATC-----TGATGA
Wiggum         GACGTTTCTACTCAAAAATACATGATGGAGATTCAGCGAGATAAGCAATT-GGATTATC-----TGATGA
PPI-Soy-FTB    CTCGCAACGCCCAAACCCTCAT-GTTGGAGCTTCAACGCGATAATCACAT-GCAGTAT-----GTCTCCA
DuP-Soy-FTB    CTCGCAACGCCCAAACCCTCAT-GTTGGAGCTTCAACGCGATAATCACAT-GCAGTAT-----GTCTCCA
PPI-Corn-FTB   GCGCCCAACACGAAATCCATCATGCTAGAGCTGTGGCGTGATCAGCATAT-CGAGTATC-----TGACGC
DuP-Corn-FTB   GCGCCCAACACGAAATCCATCATGCTAGAGCTGTGGCGTGATCAGCATAT-CGAGTATC-----TGACGC
Pea FT-B       ACCGCGGCGGAGACACCAACTCCGACGGTGAGTCAGAGAGATCAATGGATAGTAGAATCACAGGTCTTTC
Tomato         TCCCCCAACTCTCCGTCCGACCTCATAGAGATCGAACGTGACAAACACTT-CGGTTATC-----TAAGCC
Tobacco        CCNCCCAATTC---------CCACTTAGAGACTTCAACAGAAAAGCACTT-CGATTATC-----TCACTC

                        290       300       310       320       330       340       350
               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
PPI-BnFTb      --------------------------------------------------------TGGCTTTGTTACTG
era1           AAGGCTTAAGGCAGCTT---GGTCCGCAGTTTTCTTCCTTAGATGCTAATCGACCTTGGCTTTGTTACTG
Wiggum         AAGGCTTAAGGCAGCTT---GGTCCGCAGTTTTCTTCCTTAGATGCTAATCGACCTTGGCTTTGTTACTG
PPI-Soy-FTB    AAGGCCTTCGCCATCTC---AGTTCCGCATTTTCCGTTTTGGACGCTAATCGACCCTGGCTCTGCTACTG
DuP-Soy-FTB    AAGGCCTTCGCCATCTC---AGTTCCGCATTTTCCGTTTTGGACGCTAATCGACCCTGGCTCTGCTACTG
PPI-Corn-FTB   CTGGGCTGAGGCATATG---GGACCAGCCTTTCATGTTCTAGATGCCAATCGCCCTTGGCTATGCTACTG
DuP-Corn-FTB   CTGGGCTGAGGCATATG---GGACCAGCCTTTCATGTTCTAGATGCCAATCGCCCTTGGCTATGCTACTG
Pea FT-B       ATATTTATCAACTCTTCGCCAATATTCCTCCTAACGCCCAATCTATCATTCGACCTTGGCTGTGTTACTG
Tomato         AAGGTCTCAGAAAACTT---GGTCCGTCGTTTTCCGTTTTGGATGCCAGTCGACCATGGCTTTGCTACTG
Tobacco        GAGGTCTCAGAAAACTT---GGTCCGTCGTTCTCCGTCTTGGATGCTAATCGACCATGGCTTTGCTACTG

                        360       370       380       390       400       410       420
               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
PPI-BnFTb      GATTCTTCATTCAATTGCTTTGCTTGGGGAGTCTGTGGATGATGACTTAGAAAACAATGCAATCGATTTT
era1           GATTCTTCATTCAATAGCTTTGCTTGGGGAGACTGTGGATGATGAATTAGAAAGCAATGCCATTGACTTC
Wiggum         GATTCTTCATTCAATAGCTTTGCTTGGGGAGACTGTGGATGATGAATTAGAAAGCAATGCCATTGACTTC
PPI-Soy-FTB    GATCTTCCACTCCATTGCTTTGTTGGGAGAATCCGTCGATGATGAACTCGAAGATAACGCTATCGATTTT
DuP-Soy-FTB    GATCTTCCACTCCATTGCTTTGTCGGGAGAATCCGTCGATGATGAACTCGAAGATAACGCTATCGATTTT
PPI-Corn-FTB   GATGGTTCATCCACTTGCTTTGCTGGATGAAGCACTTGATGATGATCTTGAGAATGATATCATAGACTTC
DuP-Corn-FTB   GATGGTTCATCCACTTGCTTTGCTGGATGAAGCACTTGATGATGATCTTGAGAATGATATCATAGACTTC
Pea FT-B       GATTATTCATTCAATTGCTTTGTTGGGAGAATCTATTGATGATGATCTCGAAGATAACACTGTCGATTTT
Tomato         GACACTTCATTCAATCGCTTTGTTGGGAGAATCTATTGGTGGCAAACTGGAAAATGATGCAATTGACTTT
Tobacco        GATACTTCATTCAATCGCTTTGTTGGGAGAATCTATTGATGCCCAACTGGAAAATGATGCAATTGACTTT

                        430       440       450       460       470       480       490
               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
PPI-BnFTb      CTTGGACGTTGCCAGGGTTCTGATGGTGGATATGGTGGTGGTCCTGGCCAACTTCCACATCTTGCAACAA
era1           CTTGGACGCTGCCAGGGCTCTGAAGGTGGATACGGTGGTGGTCCTGGCCAACTTCCACATCTTGCAACTA
Wiggum         CTTGGACGCTGCCAGGGCTCTGAAGGTGGATACGGTGGTGGTCCTGGCCAACTTCCACATCTTGCAACTA
PPI-Soy-FTB    CTTAACCGTTGCCAGGATCCGAATGGTGGATATGCCGGGGGACCAGGCCAGATGCCTCATATTGCCACAA
DuP-Soy-FTB    CTTAACCGTTGCCAGGATCCGAATGGTGGATATGCCGGGGGACCAGGCCAGATGCCTCATATTGCCACAA
PPI-Corn-FTB   TTAGCTCGATGTCAGGATAAAGATGGTGGATATAGTGGTGGACCTGGACAGTTGCCTCACCTAGCTACGA
DuP-Corn-FTB   TTAGCTCGATGTCAGGATAAAGATGGTGGATATAGTGGTGGACCTGGACAGTTGCCTCACCTAGCTACGA
Pea FT-B       CTTAACCGTTGCCAGGATCCAAATGGTGGATATGCTGGGGGACCTGGTCAGATGCCTCATCTTGCCACAA
Tomato         CTGACCCGTTGCCAGGATAAAGATGGTGGCTATGGAGGTGGACCTGGTCAGATGCCTCATCTTGCAACTA
Tobacco        CTGAGCCGTTGCCAGGATGAAGATGGTGGCTATGGTGGTGGACCTGGTCAGATGCCTCATCTTGCAACTA

                        500       510       520       530       540       550       560
               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
PPI-BnFTb      GTTATGCTGCAGTGAATACACTTGTTACTTTAGGAGGTGAGAAAGCCTTCTCTTCAATTAACAGAGAACA
era1           CTTATGCTGCAGTGAATGCACTTGTTACTTTAGGAGGTGACAAAGCCCTTTCTTCAATTAATAGAGAAAA
Wiggum         CTTATGCTGCAGTGAATGCACTTGTTACTTTAGGAGGTGACAAAGCCCTTTCTTCAATTAATAGAGAAAA
PPI-Soy-FTB    CTTATGCTGCTGTTAATTCACTTATTACTTTGGGTGGTGAGAAATCCCTGGCATCAATTAATAGAGATAA
DuP-Soy-FTB    CTTATGCTGCTGTTAATTCACTTATTACTTTGGGTGGTGAGAAATCCCTGGCATCAATTAATAGAGATAA
PPI-Corn-FTB   CTTATGCTGCTGTAAATACACTTGTGACAATAGGGAGCGAAAGAGCATTGTCATCAATCAATAGGGGCAA
DuP-Corn-FTB   CTTATGCTGCTGTAAATACACTTGTGACAATAGGGAGCCAAAGAGCATTGTCATCAATCAATAGGGGCAA
Pea FT-B       CTTATGCTGCAGTCAATACTCTTATTACTCTGGGTGGTGAGAAATCTTTGGCATCTATTAATAGAAATAA
Tomato         CTTATGCTGCAGTCAATTCACTAATAACTTTGGGCAAACCTGAAGCTCTGTCATCAATTAATAGAGAAAA
Tobacco        CTTATGCTGCAGTCAATTCACTCATAACTTTGGGCAGCCCTAAAGCTCTGTCATCAATCAATAGAGAAAA

                        570       580       590       600       610       620       630
               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
PPI-BnFTb      AATGGCTTGTTTCTTAAGACGAATGAAGGATACAAATGGAGGTTTCAGGATGCATAATATGGGAGAAATA
era1           AATGTCTTGTTTTTTAAGACGGATGAAGGATACAAGTGGAGGTTTCAGGATGCATGATATGGGAGAAATT
Wiggum         AATGTCTTGTTTTTTAAGACGGATGAAGGATACAAGTGGAGGTTTCAGGATGCATGATATGGGAGAAATG
PPI-Soy-FTB    ACTGTATGGGTTTCTGCGGCGGATGAAGCAACCAAATGGTGGATTCAGGATGCATGATGAAGGTGAAATT
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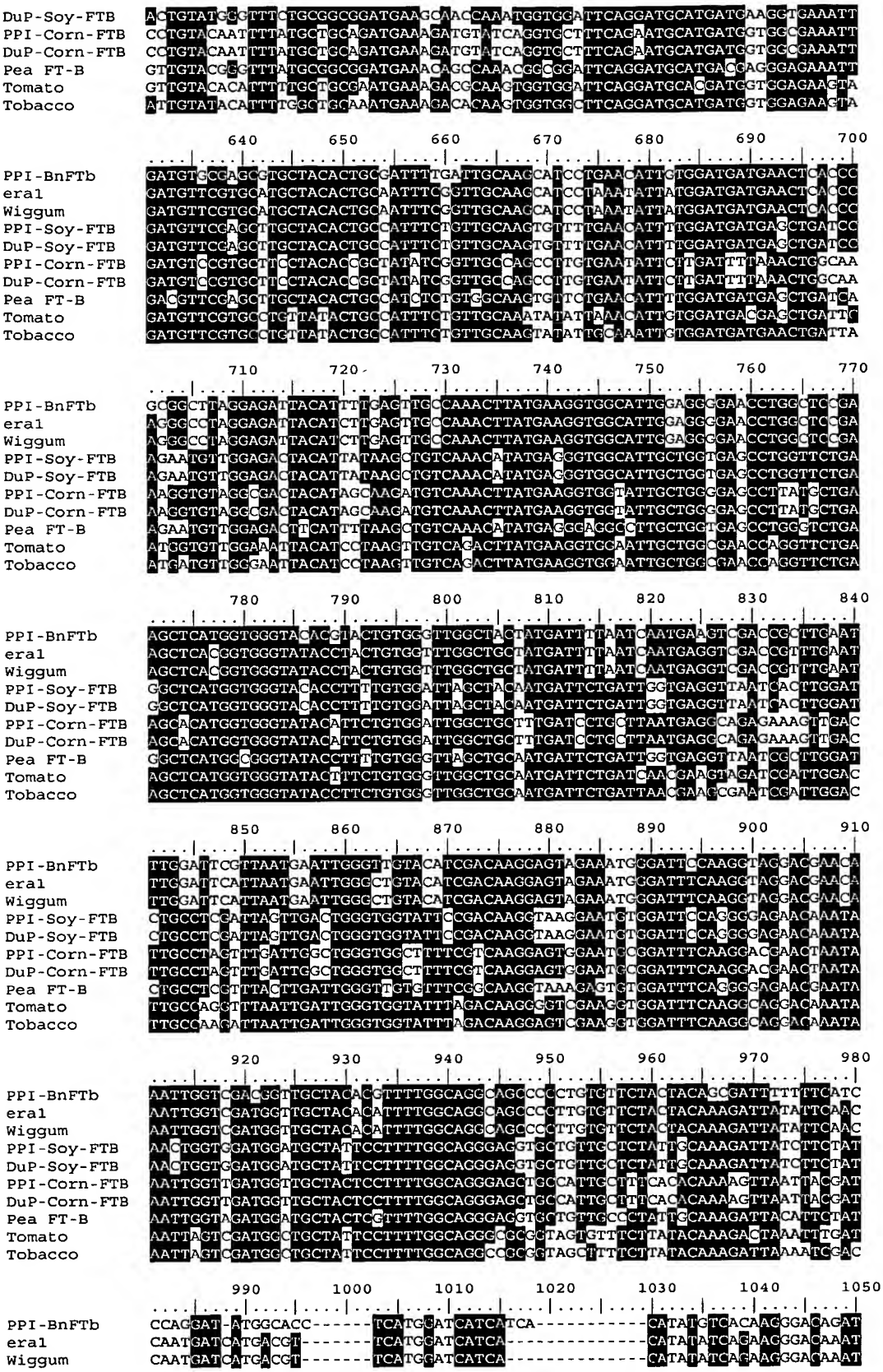
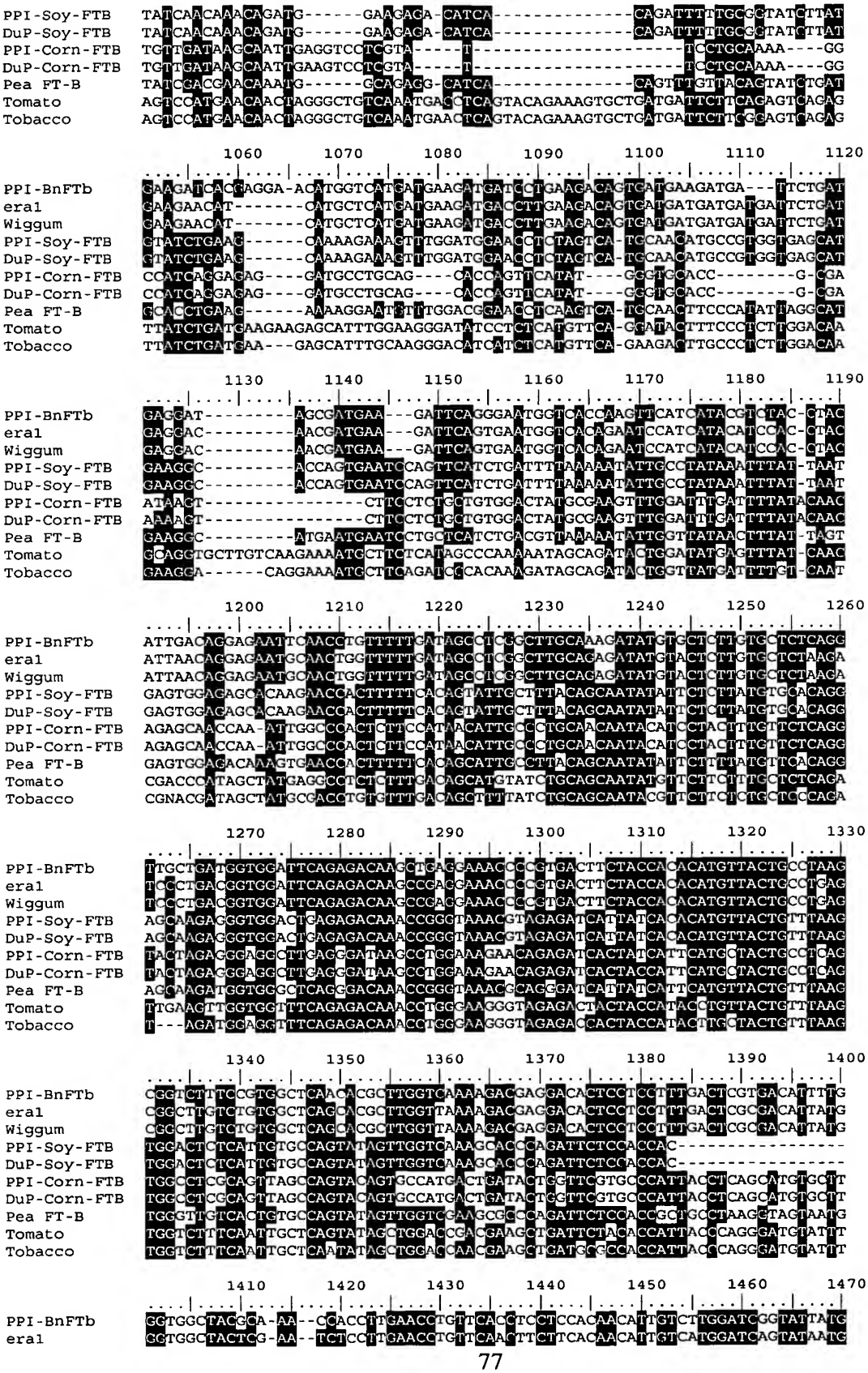
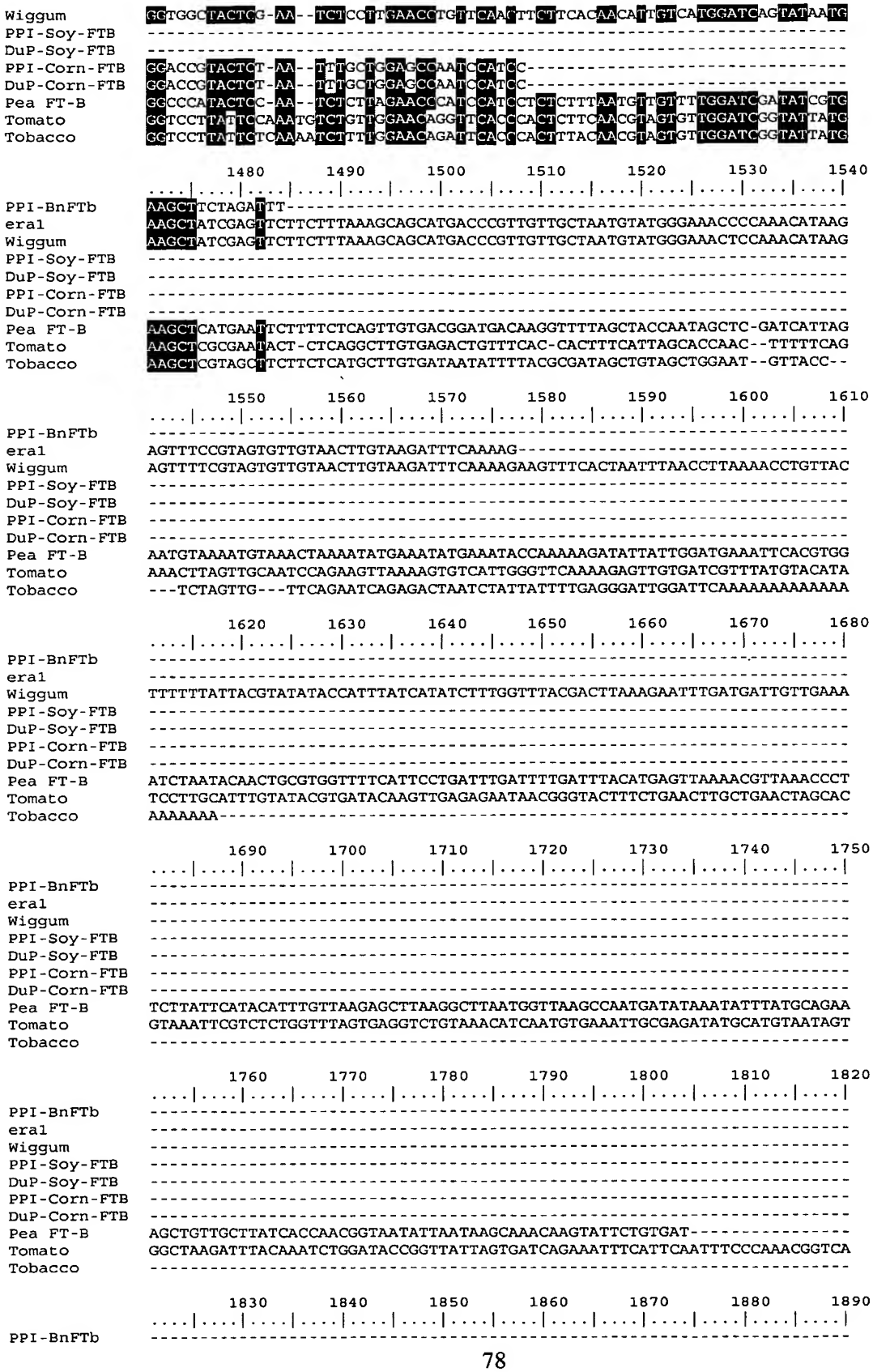

DuP-Soy-FTB
PPI-Corn-FTB
DuP-Corn-FTB
Pea FT-B
Tomato
Tobacco
640 650 660 670 680 690 700
PPI-BnFTb
era1
Wiggum
PPI-Soy-FTB
DuP-Soy-FTB
PPI-Corn-FTB
DuP-Corn-FTB
Pea FT-B
Tomato
Tobacco
710 720 730 740 750 760 770
PPI-BnFTb
era1
Wiggum
PPI-Soy-FTB
DuP-Soy-FTB
PPI-Corn-FTB
DuP-Corn-FTB
Pea FT-B
Tomato
Tobacco
780 790 800 810 820 830 840
PPI-BnFTb
era1
Wiggum
PPI-Soy-FTB
DuP-Soy-FTB
PPI-Corn-FTB
DuP-Corn-FTB
Pea FT-B
Tomato
Tobacco
850 860 870 880 890 900 910
PPI-BnFTb
era1
Wiggum
PPI-Soy-FTB
DuP-Soy-FTB
PPI-Corn-FTB
DuP-Corn-FTB
Pea FT-B
Tomato
Tobacco
920 930 940 950 960 970 980
PPI-BnFTb
era1
Wiggum
PPI-Soy-FTB
DuP-Soy-FTB
PPI-Corn-FTB
DuP-Corn-FTB
Pea FT-B
Tomato
Tobacco
990 1000 1010 1020 1030 1040 1050
PPI-BnFTb
era1
Wiggum


PPI-Soy-FTB
DuP-Soy-FTB
PPI-Corn-FTB
DuP-Corn-FTB
Pea FT-B
Tomato
Tobacco
1060 1070 1080 1090 1100 1110 1120
PPI-BnFTb
era1
Wiggum
PPI-Soy-FTB
DuP-Soy-FTB
PPI-Corn-FTB
DuP-Corn-FTB
Pea FT-B
Tomato
Tobacco
1130 1140 1150 1160 1170 1180 1190
PPI-BnFTb
era1
Wiggum
PPI-Soy-FTB
DuP-Soy-FTB
PPI-Corn-FTB
DuP-Corn-FTB
Pea FT-B
Tomato
Tobacco
1200 1210 1220 1230 1240 1250 1260
PPI-BnFTb
era1
Wiggum
PPI-Soy-FTB
DuP-Soy-FTB
PPI-Corn-FTB
DuP-Corn-FTB
Pea FT-B
Tomato
Tobacco
1270 1280 1290 1300 1310 1320 1330
PPI-BnFTb
era1
Wiggum
PPI-Soy-FTB
DuP-Soy-FTB
PPI-Corn-FTB
DuP-Corn-FTB
Pea FT-B
Tomato
Tobacco
1340 1350 1360 1370 1380 1390 1400
PPI-BnFTb
era1
Wiggum
PPI-Soy-FTB
DuP-Soy-FTB
PPI-Corn-FTB
DuP-Corn-FTB
Pea FT-B
Tomato
Tobacco
1410 1420 1430 1440 1450 1460 1470
PPI-BnFTb
era1


Wiggum
PPI-Soy-FTB
DuP-Soy-FTB
PPI-Corn-FTB
DuP-Corn-FTB
Pea FT-B
Tomato
Tobacco
1480 1490 1500 1510 1520 1530 1540
PPI-BnFTb
era1
Wiggum
PPI-Soy-FTB
DuP-Soy-FTB
PPI-Corn-FTB
DuP-Corn-FTB
Pea FT-B
Tomato
Tobacco
1550 1560 1570 1580 1590 1600 1610
PPI-BnFTb
era1
Wiggum
PPI-Soy-FTB
DuP-Soy-FTB
PPI-Corn-FTB
DuP-Corn-FTB
Pea FT-B
Tomato
Tobacco
1620 1630 1640 1650 1660 1670 1680
PPI-BnFTb
era1
Wiggum
PPI-Soy-FTB
DuP-Soy-FTB
PPI-Corn-FTB
DuP-Corn-FTB
Pea FT-B
Tomato
Tobacco
1690 1700 1710 1720 1730 1740 1750
PPI-BnFTb
era1
Wiggum
PPI-Soy-FTB
DuP-Soy-FTB
PPI-Corn-FTB
DuP-Corn-FTB
Pea FT-B
Tomato
Tobacco
1760 1770 1780 1790 1800 1810 1820
PPI-BnFTb
era1
Wiggum
PPI-Soy-FTB
DuP-Soy-FTB
PPI-Corn-FTB
DuP-Corn-FTB
Pea FT-B
Tomato
Tobacco
1830 1840 1850 1860 1870 1880 1890
PPI-BnFTb

```
era1            ------------------------------------------------------------------------
Wiggum          ------------------------------------------------------------------------
PPI-Soy-FTB     ------------------------------------------------------------------------
DuP-Soy-FTB     ------------------------------------------------------------------------
PPI-Corn-FTB    ------------------------------------------------------------------------
DuP-Corn-FTB    ------------------------------------------------------------------------
Pea FT-B        ------------------------------------------------------------------------
Tomato          CCTAAGTTTAGGATATTGCTTTAAAATATTATTTATTTTTCATTTAAGAATCAAAAAAAAAAAAAAAAAA
Tobacco         ------------------------------------------------------------------------

                ....|....
PPI-BnFTb       ---------
era1            ---------
Wiggum          ---------
PPI-Soy-FTB     ---------
DuP-Soy-FTB     ---------
PPI-Corn-FTB    ---------
DuP-Corn-FTB    ---------
Pea FT-B        ---------
Tomato          AAAAAAAAA
Tobacco         ---------
```

Table 10D. ClustalW Amino Acid Analysis of FT Beta Subunits

1) PPI-BnFTB; FT3 (SEQ ID NO:15)
2) era1 (SEQ ID NO:2)
3) Wiggum (SEQ ID NO:87)
4) PPI-Soy-FTB; FT5 (SEQ ID NO:42)
5) DuP-Soy-FTB (SEQ ID NO:88)
6) PPI-Corn-FTB; FT6 (SEQ ID NO:45)
7) DuP-Corn-FTB (SEQ ID NO:89)
8) Pea-FT-B (SEQ ID NO:90)
9) Tomato (SEQ ID NO:91)
10) Tobacco (SEQ ID NO:92)

```
                        10        20        30        40        50        60        70
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
PPI-BnFTB       ------------------------------------------------------------------------
era1            ------------------------------------------------------------------------
Wiggum          MPVVTRLIRLKCVGLRLDRSGLNRRICHGGHGESTRRRVMEELSSLTVSQREQFLVENDVFGIYNYFDAS
PPI-Soy-FTB     ------------------------------------------------------------------ATI
DuP-Soy-FTB     ------------------------------------------------------------------ATI
PPI-Corn-FTB    ----------------------------------ADPDLPRLTVTQVEQMKVEARVGDIYRSLFGA
DuP-Corn-FTB    ----------------------------------ADPDLPRLTVTQVEQMKVEARVGDIYRSLFGA
Pea FT-B        ------------------------------------------------------------MEA
Tomato          ------------------------------------MESRKVTKTLEDQWVVERRVREIYDYFYSI
Tobacco         ------------------------------------GTSGTRTLEDQWMVERQVREIYNFFYSI

                        80        90        100       110       120       130       140
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
PPI-BnFTB       ------------------------------------------------WLCYWILHSIALLGESVDDDLENNAI
era1            --------MEIQRDKQLDYLMKGLRQLGPQFSSLDAN-----RPWLCYWILHSIALLGETVDDELESNAI
Wiggum          DVSTQKYMMEIQRDKQLDYLMKGLRQLGPQFSSLDAN-----RPWLCYWILHSIALLGETVDDELESNAI
PPI-Soy-FTB     PRNAQTLMLELQRDNHMQYVSKGLRHLSSAFSVLDAN-----RPWLCYWIFHSIALLGESVDDELEDNAI
DuP-Soy-FTB     PRNAQTLMLELQRDNHMQYVSKGLRHLSSAFSVLDAN-----RPWLCYWIFHSIALSGESVDDELEDNAI
PPI-Corn-FTB    APNTKSIMLELWRDQHIEYLTPGLRHMGPAFHVLDAN-----RPWLCYWMVHPVALLDEALDDDLENDII
DuP-Corn-FTB    APNTKSIMLELWRDQHIEYLTPGLRHMGPAFHVLDAN-----RPWLCYWMVHPVALLDEALDDDLENDII
Pea FT-B        STAAETPTPTVSGRDQWIVESQ-VFHTYQLFANIPPNAQSII RPWLCYWIIHSIALLGESIDDDLEENTV
Tomato          SPNSPSDLTEIERDKHFGYLSQGLRKLGPSFSVLDAS-----RPWLCYWTLHSIALLGESIGGKLENEAI
Tobacco         PPNS---HLETSTEKHFDYLTPGLRKLGPSFSVLDAN-----RPWLCYWILHSIALLGESIDAQLENDAI

                        150       160       170       180       190       200       210
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
PPI-BnFTB       DFLGRCQGSDGGYCGGPGQLPHLATSYAAVNTLVTLGGEKAFSSINREQMACFLRRMKDTNGGFRMHNMG
era1            DFLGRCQGSEGGYCGGPGQLPHLATTYAAVNALVTLGGDKALSSINREKMSCFLRRMKDTSGGFRMHDMG
Wiggum          DFLGRCQGSEGGYCGGPGQLPHLATTYAAVNALVTLGGEKALSSINREKMSCFLRRMKDTSGGFRMHDMG
PPI-Soy-FTB     DFLNRCQDPNGGYAGGPGQMPHIATTYAAVNSLITLGGEKSLASINRDKLYGFLRRMKQPNGGFRMHDEG
DuP-Soy-FTB     DFLNRCQDPNGGYAGGPGQMPHIATTYAAVNSLITLGGEKSLASINRDKLYGFLRRMKQPNGGFRMHDEG
PPI-Corn-FTB    DFLARCQDKDGGYSGGPGQLPHLATTYAAVNTLVTIGSERALSSINRGNLYNFMLQMKDVSGAFRMHDGG
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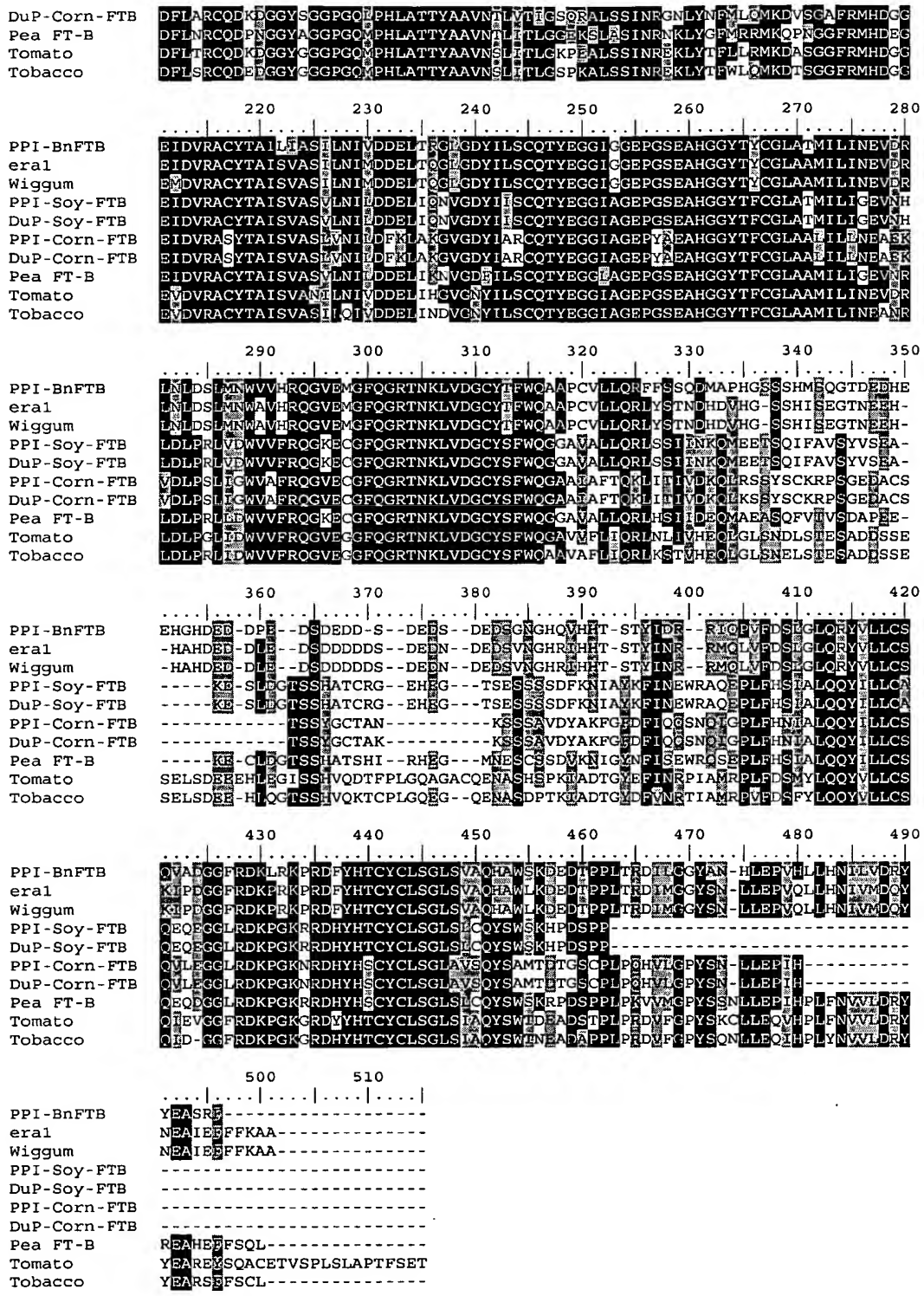


Also included in the invention is the farnesyl transferase alpha consensus sequence of SEQ ID NO:93 and the farnesyl transferase beta consensus sequence of SEQ ID NO:94 To generate the consensus sequence, the farnesyl transferase alpha and farnesyl transferase beta

sequences of the invention were aligned using the program BioEdit. The homology between the farnesyl transferase alpha (FTA) polypeptide sequences of the invention is shown graphically in the ClustalW analysis shown in Table 10E. The homology between the farnesyl transferase beta (FTB) polypeptide sequences of the invention is shown graphically in the ClustalW analysis shown in Table 10F.

Table 10E ClustalW Amino Acid Analysis of FT Alpha

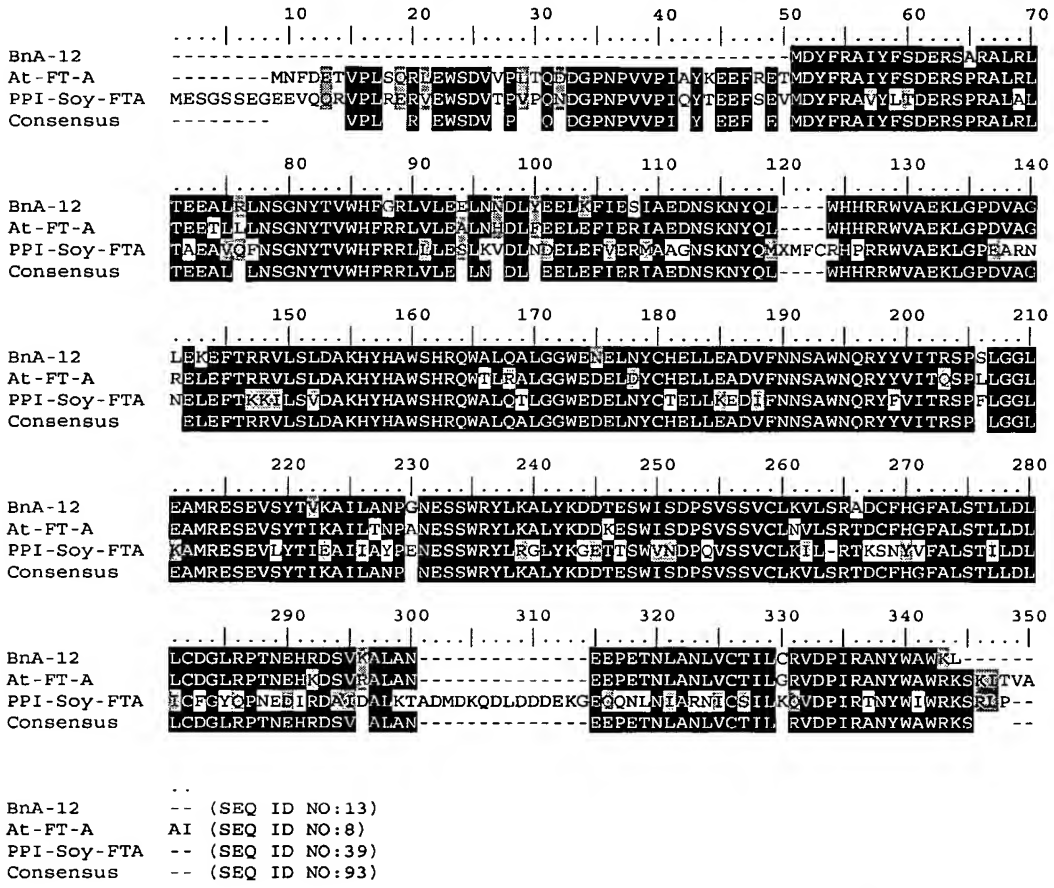
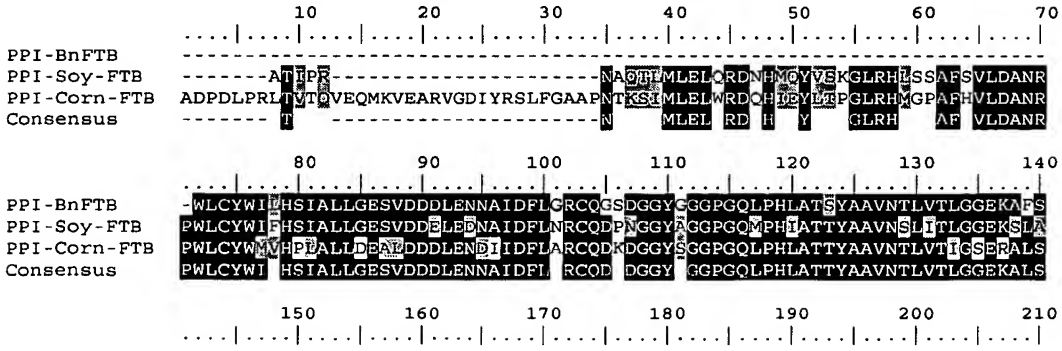
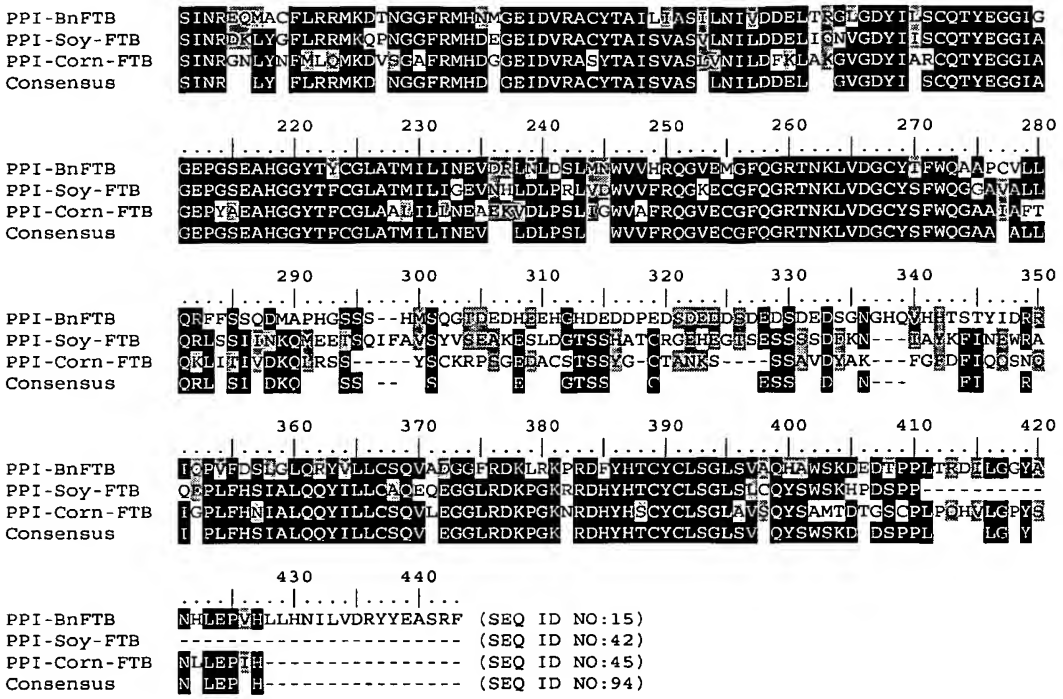


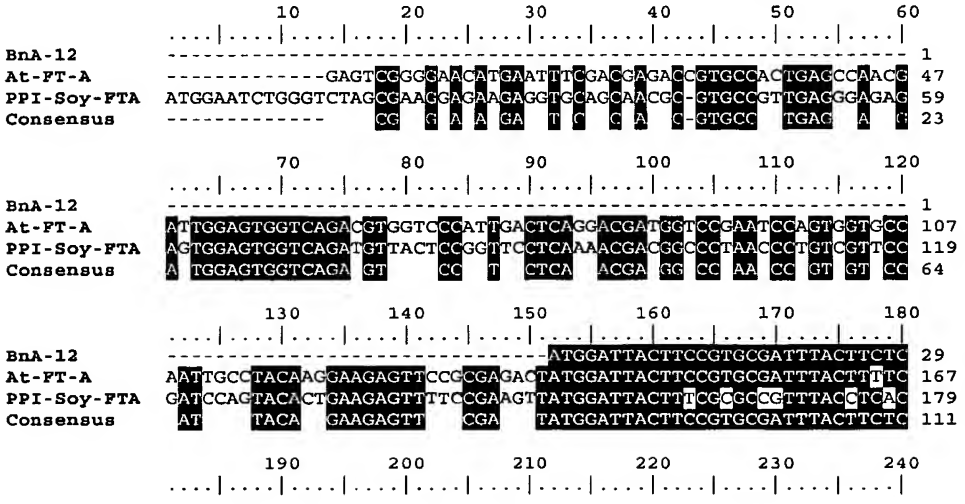
Table 10F ClustalW Amino Acid Analysis of FT Beta

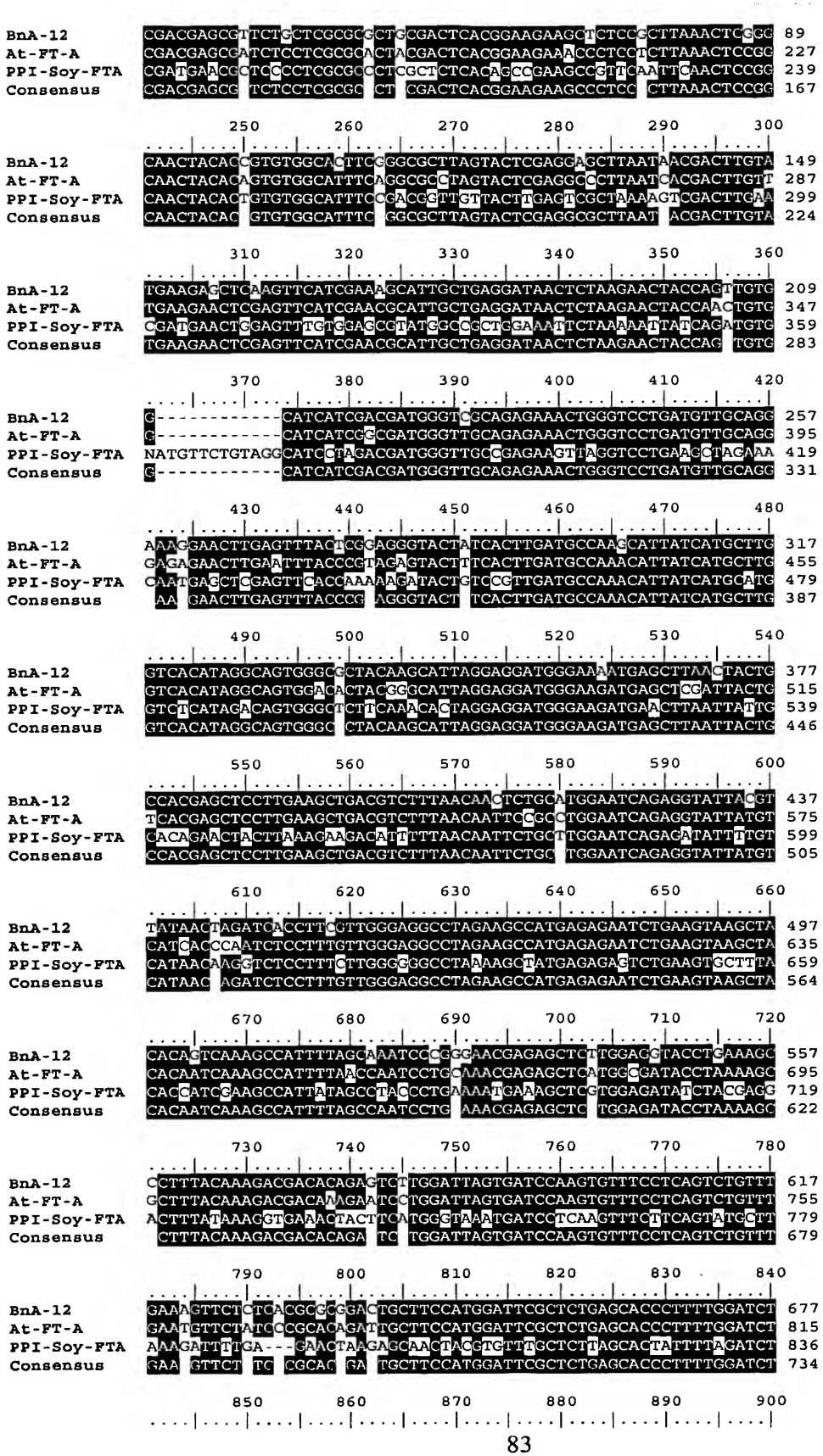




Also included in the invention is the farnesyl transferase alpha consensus sequence of SEQ ID NO:95 and the farnesyl transferase beta consensus sequence of SEQ ID NO:96. To generate the consensus sequence, the farnesyl transferase alpha and farnesyl transerase beta sequences of the invention were aligned using the program BioEdit. The homology between the farnesyl transferase alpha (FTA) nucleic acid sequences of the invention is shown graphically in the ClustalW analysis shown in Table 10G. The homology between the farnesyl transferase beta (FTB) nucleic acid sequences of the invention is shown graphically in the ClustalW analysis shown in Table 10H.

Table 10G ClustalW Nucleic Acid Analysis of FT Alpha




BnA-12
At-FT-A
PPI-Soy-FTA
Consensus

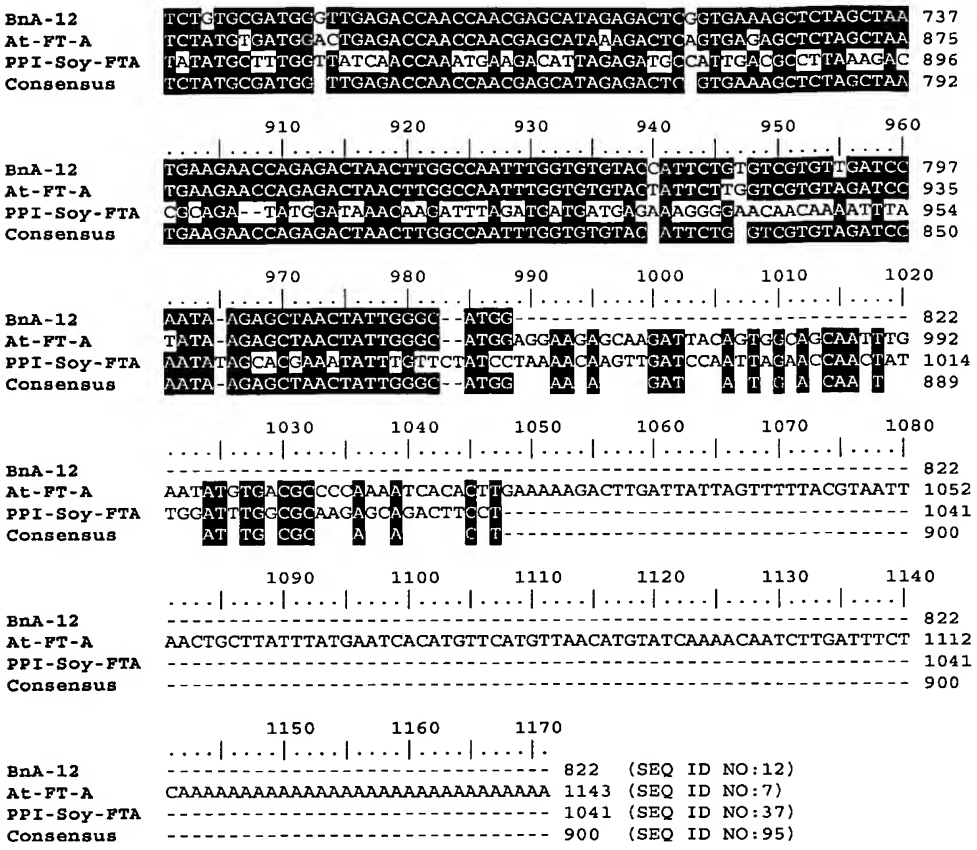
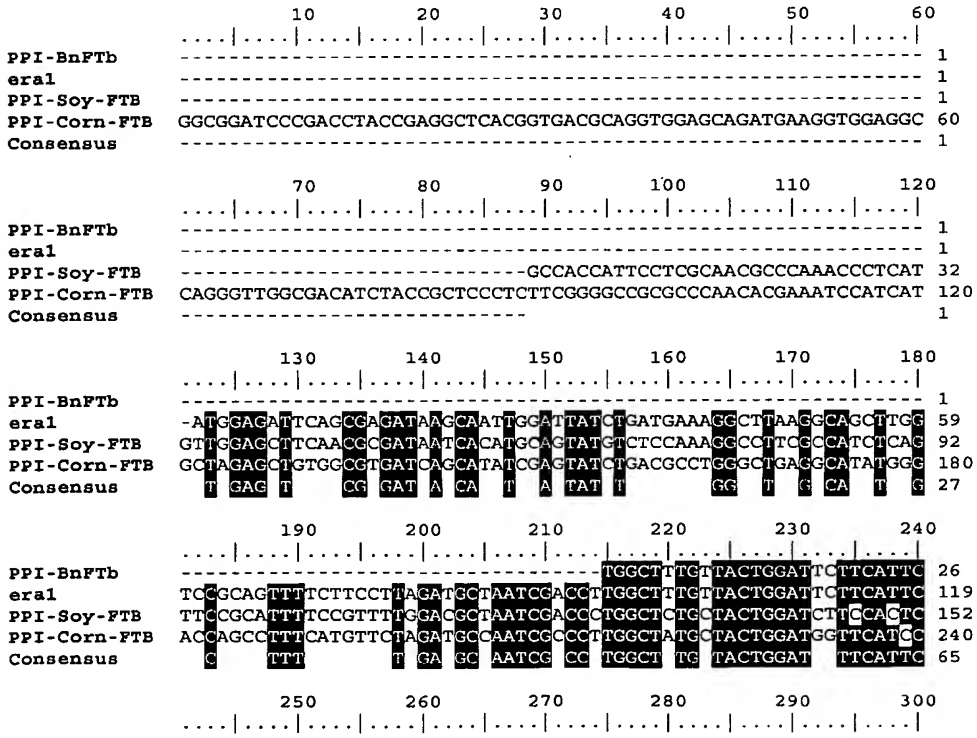
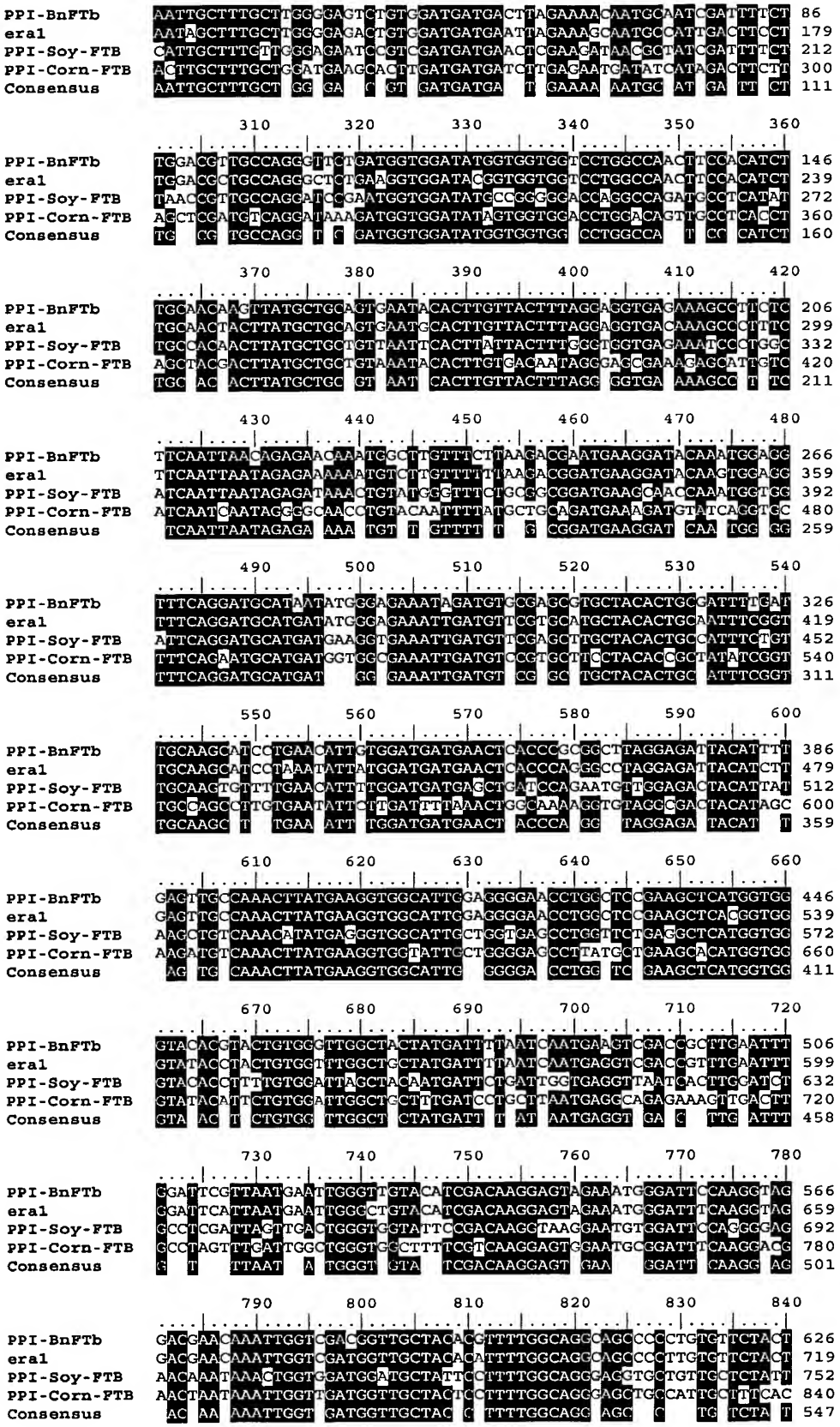
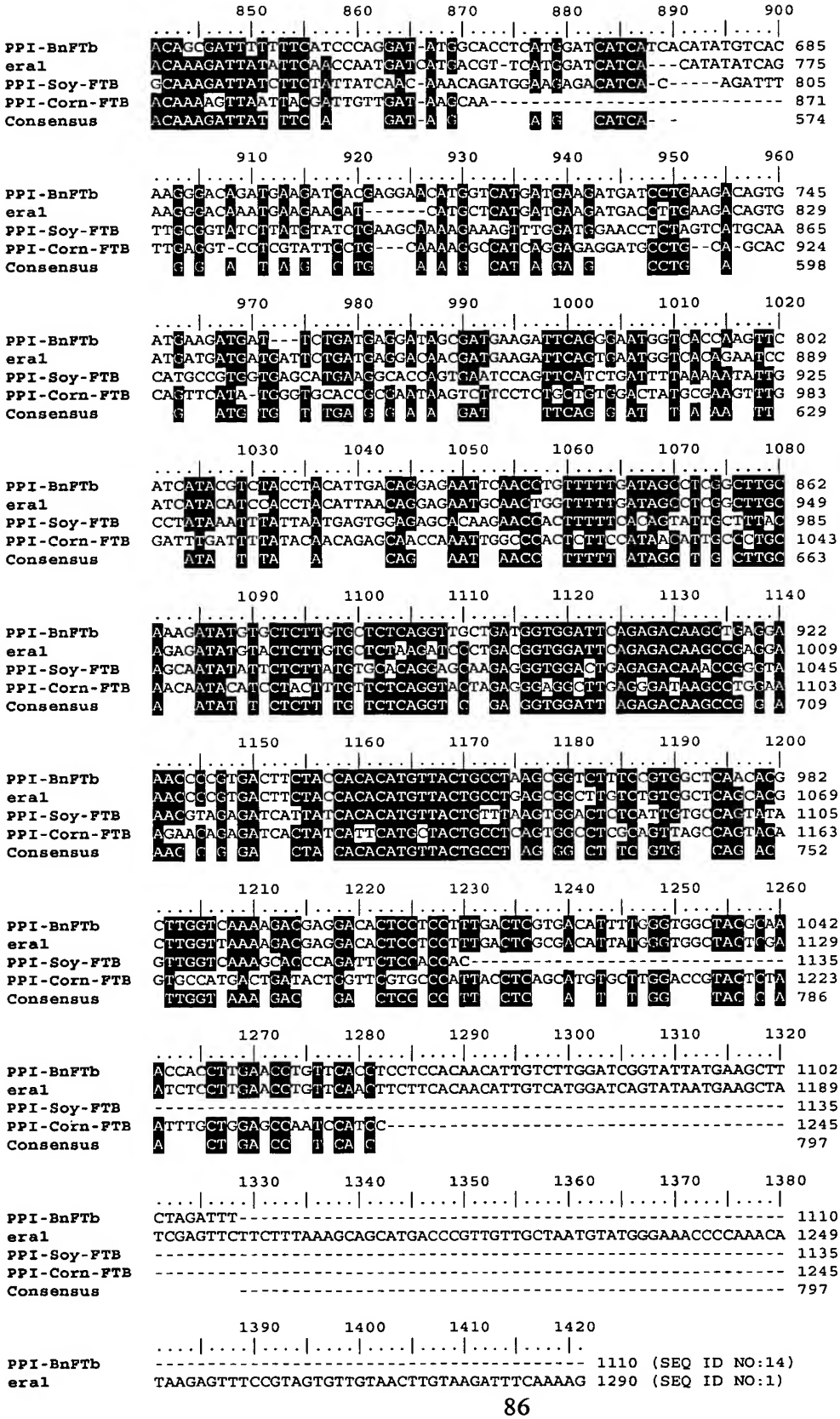


Table 10H ClustalW Nucleic Acid Analysis of FT Beta




PPI-BnFTb
era1
PPI-Soy-FTB
PPI-Corn-FTB
Consensus


PPI-BnFTb
era1
PPI-Soy-FTB
PPI-Corn-FTB
Consensus
(SEQ ID NO:14)
(SEQ ID NO:1)

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PPI-Soy-FTB    ---------------------------------------- 1135 (SEQ ID NO:40)
PPI-Corn-FTB   ---------------------------------------- 1245 (SEQ ID NO:43)
Consensus      ---------------------------------------- 797  (SEQ ID NO:97)
```

Example 13: Vector constructs for Transformation

The FTA or FTB sequences have be used to produce constructs suitable for transformation into plants and under the control of appropriate regulatory sequences. The gene sequences were in either the sense orientation for over-expression or the antisense orientation for down-regulation. Portions of these sequences have been used to construct a double-stranded-RNA-inhibition (dsRNAi) construct. A sequence of preferably not less than 21 nt was cloned as an inverse repeat separated by a linker that when expressed results in down-regulation of the target gene. Double antisense (DA) vectors have been created in which a direct repeat of an antisense sequence is separated by a spacer sequence such as GUS. Promoters have been used for constitutive expression such as the 35S CaMV promoter, the MuA *Zea maize* promoter or inducible by specific environmental or cellular cues such as the ABA levels or drought conditions which induce expression of the RD29A promoter. Alternatively, tissue or organelle specific promoters such as the HIC or CUT1 promoter can be used. Such constructs have been transformed into *Arabidopsis thaliana*, *Brassica*, *Zea maize*, *Glycine max*. Other species can be transformed as desired. Each species to be transformed may make use of specific regulatory sequences as appropriate for those particular species. Transformed plants have be selected and their phenotypic properties analyzed. The transgenic plants were assessed for characteristics such as increased tolerance to drought, altered biomass accumulation, yield, nutritional requirements such as minerals or micro-nutrients, biotic stress such as fungal, bacterial, or other such pathogen infection or attack or any other such physical or biochemical characteristic.

Example 14: Plant Transformation

Arabidopsis thaliana transgenic plants were made by flower dipping method into an *Agrobacterium* culture. Wild type plants were grown under standard conditions until they began flowering. The plant was inverted for 2 min into a solution of *Agrobacterium* culture. Plants were then bagged for two days to maintain humidity and then uncovered to continue growth and seed development. Mature seed was bulk harvested.

Transformed T1 plants were selected by germination and growth on MS plates containing 50 μg/ml kanamycin. Green, kanamycin resistant seedlings were identified after 2 weeks growth and transplanted to soil. Plants were bagged to ensure self fertilization and the T2

seed of each plant harvested separately. During growth of T1 plants leaf samples were harvested, DNA extracted and Southern analysis performed.

T2 seeds were analyzed for Kan^R segregation. From those lines that showed a 3:1 resistant phenotype surviving T2 plants were grown, bagged during seed set, and T3 seed harvested from each line. T3 seed was again used for Kan^R segregation analysis and those lines showing 100% Kan^R phenotype were selected as homozygous lines. Further analysis was done using T3 seed.

Transgenic *Brassica napus* plants were produced using *Agrobacterium* mediated transformation of cotyledon petiole tissue. Seeds were sterilized as follows. Seeds were wetted with 95% ethanol for a short period of time such as 15 seconds. Approximately 30 ml of sterilizing solution I was added (70% Javex , 100µl Tween20) and left for approximately 15 minutes. Solution I was removed and replaced with 30 ml of solution II (0.25% mecuric chloride, 100µl Tween20) and incubated for about 10 minutes. Seeds were rinsed with at least 500 ml double distilled sterile water and stored in a sterile dish. Seeds were germinated on plates of $^1/_2$ MS medium, pH 5.8, supplemented with 1% sucrose and 0.7% agar. Fully expanded cotyledons were harvested and placed on Medium I (Murashige minimal organics (MMO), 3% sucrose, 4.5 mg/L benzyl adenine (BA), 0.7% phytoagar, pH5.8). An *Agrobacterium* culture containing the nucleic acid construct of interest was grown for 2 days in AB Minimal media. The cotyledon explants were dipped such that only the cut portion of the petiole is contacted by the *Agrobacterium* solution. The explants were then embedded in Medium I and maintained for 5 days at 24°C, with 16,8 hr light dark cycles. Explants were transferred to Medium II (Medium I, 300 mg/L timentin,) for a further 7 days and then to Medium III (Medium II, 20 mg/L kanamycin). Any root or shoot tissue which had developed at this time was dissected away. Transfer explants to fresh plates of Medium III after 14 -21 days. When regenerated shoot tissue developed the regenerated tissue was transferred to Medium IV (MMO, 3% sucrose, 1.0% phytoagar, 300 mg/L timentin, 20 mg/L 20 mg/L kanamycin). Once healthy shoot tissue developed shoot tissue dissected from any callus tissue was dipped in 10X IBA and transferred to Medium V (Murashige and Skooge (MS), 3% sucrose, 0.2 mg/L indole butyric acid (IBA), 0.7% agar, 300 mg/L timentin, 20 mg/L 20 mg/L kanamycin) for rooting. Healthy plantlets were transferred to soil.

Transgenic *Glycine max*, *Zea maize* and cotton can be produced using *Agrobacterium*-based methods which are known to one of skill in the art. Alternatively one can use a particle or non-particle biolistic bombardment transformation method. An example of non-particle biolistic

transformation is given in U.S. Patent Application 20010026941. Viable plants are propogated and homozygous lines are generated. Plants are tested for the presence of drought tolerance, physiological and biochemical phenotypes as described elsewhere.

The following table indentifies the constructs and the species which they have been transformed.

Table 11.

SEQ ID NO:	SEQ	Species Transformed			
SEQ ID NO:10	pBI121-35S-anti-AtFTA	Arabidopsis thaliana			
SEQ ID NO:46	pBI121-35S-AtFTA	Arabidopsis thaliana	Brassica napus		
SEQ ID NO:47	pBI121-rd29A-anti-AtFTA	Arabidopsis thaliana	Brassica napus		
SEQ ID NO:48	pBI121-35S-DA-AtFTA	Arabidopsis thaliana	Brassica napus		
SEQ ID NO:49	pBI121-RD29A-DA-AtFTA	Arabidopsis thaliana	Brassica napus		
SEQ ID NO:50	MuA-anti-GmFTA				Glycine max
SEQ ID NO:51	RD29A-anti-GmFTA				Glycine max
SEQ ID NO:52	MuA-HP-GmFTA-Nos-Term				Glycine max
SEQ ID NO:53	RD29AP-HP-GmFTA-Nos-Term				Glycine max
SEQ ID NO:54	pBI121-35S-Anti-AtFTB	Arabidopsis thaliana	Brassica napus		
SEQ ID NO:55	pBI121-RD29AP-Anti-AtFTB	Arabidopsis thaliana	Brassica napus		
SEQ ID NO:56	pBI121-35S-HP-AtFTB	Arabidopsis thaliana	Brassica napus		
SEQ ID NO:57	pBI121-RD29AP-HP-AtFTB	Arabidopsis thaliana	Brassica napus		
SEQ ID NO:58	pBI121-35S-AtFTB	Arabidopsis thaliana			
SEQ ID NO:59	MuA-anti-GmFTB-Nos-Term				Glycine max
SEQ ID NO:60	RD29AP-anti-GmFTB-Nos-Term				Glycine max
SEQ ID NO:61	MuA-HP-GmFTB-Nos-Term				Glycine max
SEQ ID NO:62	RD29AP-HP-GmFTB-Nos-Term				Glycine max
SEQ ID NO:63	MuA-anti-Zea maizeFTB-Nos-Term			Zea maiz	

SEQ ID NO:64	MuA-HP-Zea maizeFTB-Nos-Term	Zea maize

Non-limiting examples of vector constructs suitable for plant transformation are given in SEQ ID NO: 10, 46-64.

SEQ ID NO:10

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SEQ ID NO:10 is the nucleic acid sequence of pBI121-antisense-FTA vector construct used to transform *Arabidopsis* plants. Italicized sequences are the right and left border repeats (1-24, 5226-5230). Underlined sequence is the 35S promoter (2515-3318). Bold sequence is the anti-sense Farnesyl transferase alpha sequence (3334-4317).

SEQ ID NO:46

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(Underlined Seq: 35S promoter; Bold: AtFTA)

SEQ ID NO:47

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SEQ ID NO:50

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(Underlined MuA Promoter; Bold: *Glycine max* anti-FTA; lower case: NOS terminater Seq.)

SEQ ID NO:51

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(Underlined RD29A Promoter; Bold: *Glycine max* anti-*Glycine max* FTA; lower case: NOS terminater Seq.)

SEQ ID NO:52

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(Underlined: *Glycine max* FTA Anti-Sense section; Bold: MuA Promoter; Italics: *Glycine max* FTA Sense section; lower case: NOS terminater Seq.)

SEQ ID NO:53

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(Bold lower case: RD29A Promoter; Underline, Upper case: Antisense GmFTA; Upper case: Sense GmFTA; lower case: NOS terminater)

SEQ ID NO:54

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SEQ ID NO:59

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GGATGGAACCTCTAGTCATGCAACATGCCGTGGTGAGCATGAAGGCACCAGTGAATCCAGTTCA
TCTGATTTTAAAAATATTGCCTATAAATTTATTAATGAGTGGAGAGCACAAGAACCACTTTTTC
ACAGTATTGCTTTACAGCAATATATTCTCTTATGTGCACAGGAGCAAGAGGGTGGACTGAGAGA
CAAACCGGGTAAACGTAGAGATCATTATCACACATGTTACTGTTTAAGTGGACTCTCATTGTGC
CAGTATAGTTGGTCAAAGCACCCAGATTCTCCACCACgagctcgaatttccccgatcgttcaaa
catttggcaataaagtttcttaagattgaatcctgttgccggtcttgcgatgattatcatataa
tttctgttgaattacgttaagcatgtaataattaacatgtaatgcatgacgttatttatgagat
gggttttatgattagagtcccgcaattatacatttaatacgcgatagaaaacaaaatatagcg
cgcaaactaggataaattatcgcgcgcggtgtcatctatgttactagatcgggaattc
(Upper Case: MuA Promoter; Underlined: Antisense GmFTB; Bold: Sense GmFTB; Lower case: NOS terminater)

SEQ ID NO:62

GGAGCCATAGATGCAATTCAATCAAACTGAAATTTCTGCAAGAATCTCAAACACGGAGATCTCA
AAGTTTGAAAGAAAATTTATTTCTTCGACTCAAAACAAACTTACGAAATTTAGGTAGAACTTAT
ATACATTATATTGTAATTTTTTGTAACAAAATGTTTTTATTATTATTATAGAATTTTACTGGTT
AAATTAAAAATGAATAGAAAAGGTGAATTAAGAGGAGAGAGGAGGTAAACATTTTCTTCTATTT
TTTCATATTTTCAGGATAAATTATTGTAAAAGTTTACAAGATTTCCATTTGACTAGTGTAAATG
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AAACAATATTTAGCTCCTTTGTAAATACAAATTAATTTTCCTTCTTGACATCATTCAATTTTAA
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GAAGGATGTGCCGTTTGTTATAATAAACAGCCACACGACGTAAACGTAAAATGACCACATGATG
GGCCAATAGACATGGACCGACTACTAATAATAGTAAGTTACATTTTAGGATGGAATAAATATCA
TACCGACATCAGTTTTGAAAGAAAAGGGAAAAAAAGAAAAAATAAATAAAAGATATACTACCGA
CATGAGTTCCAAAAAGCAAAAAAAAAGATCAAGCCGACACAGACACGCGTAGAGAGCAAAATGA
CTTTGACGTCACACCACGAAAACAGACGCTTCATACGTGTCCCTTTATCTCTCTCAGTCTCTCT
ATAAACTTAGTGAGACCCTCCTCTGTTTTACTCACAAATATGCAAACTAGAAAACAATCATCAG
GAATAAAGGGTTTGATTACTTCTATTGGAAAGGTGGTGGAGAATCTGGGTGCTTTGACCAACTA
TACTGGCACAATGAGAGTCCACTTAAACAGTAACATGTGTGATAATGATCTCTACGTTTACCCG
GTTTGTCTCTCAGTCCACCCTCTTGCTCCTGTGCACATAAGAGAATATATTGCTGTAAAGCAAT
ACTGTGAAAAAGTGGTTCTTGTGCTCTCCACTCATTAATAAATTTATAGGCAATATTTTTAAAA
TCAGATGAACTGGATTCACTGGTGCCTTCATGCTCACCACGGCATGTTGCATGACTAGAGGTTC
CATCCAAACTTTCTTTTGCTTCAGATACATAAGATACCGCAAAAATCTGTGATGTCTCTTCCAT
CTGTTTGTTGATAATAGAAGATAATCTTTGCAATAGAGCAACAGCACCTCCCTGCCAAAAGGAA
TAGCATCCATCCACCAGTTTATTTGTTCTCCCCTGGAATCCACATTCCTTACCTTGTCGGAATA
CCACCCAGTCAACTAATCGAGGCAGATCCAAGTGATTAACCTCACCAATCAGAATCATTGTAGC
TAATCCACAAAAGGTGTACCCACCATGAGCCTCAGAACCAGGCTCACCAGCAATGCCACCCTCA
TATGTTTGACAGCTTATAATGTAGTCTCCAACATTCTGGATCAGCTCATCATCCAAAATGTTCA
AAACACTTGCAACAGAAATGGCAGTGTAGCAAGCTCGAACATCAATTTCACCTTCATCATGCAT
CCTGAATCCACCATTTGGTTGCTTCATCCGCCGCAGAAACCCATACAGTTTATCTCTATTAATT
GATGCCAGGGATTTCTCACCACCCAAAGTAATAAGTGAATTAACAGCAGCATAAGTTGTGGCAA
TATGAGGCATCTGGCCTGGTCCCCCGGCATATCCACCATTCGGATCCTGGCAACGGTTAAGAAA
ATCGATAGCGTTATCTTCGAGTTCATCATCGACGGATTCTCCCAACAAAGCAATGGAGTGGAAG
ATCCAGTAGCAGAGCCAGGGTCGATTAGCGTCCAAAACGGAAAATGCGGAACTGAGATGGCGAA
GGCCTTTGGAGACATACTGCATGTGATTATCGCGTTGAAGCTCCAACATGAGGGTTTGGGCGTT
GCGAGGAATGGTGGC**GGTGAGGTTAATCACTTGGATCTGCCTCGATTAGTTGACTGGGTGGTAT**
TCCGACAAGGTAAGGAATGTGGATTCCAGGGGAGAACAAATAAACTGGTGGATGGATGCTATTC
CTTTTGGCAGGGAGGTGCTGTTGCTCTATTGCAAAGATTATCTTCTATTATCAACAAACAGATG
GAAGAGACATCACAGATTTTTGCGGTATCTTATGTATCTGAAGCAAAAGAAAGTTTGGATGGAA
CCTCTAGTCATGCAACATGCCGTGGTGAGCATGAAGGCACCAGTGAATCCAGTTCATCTGATTT
TAAAAATATTGCCTATAAATTTATTAATGAGTGGAGAGCACAAGAACCACTTTTTCACAGTATT
GCTTTACAGCAATATATTCTCTTATGTGCACAGGAGCAAGAGGGTGGACTGAGAGACAAACCGG
GTAAACGTAGAGATCATTATCACACATGTTACTGTTTAAGTGGACTCTCATTGTGCCAGTATAG
TTGGTCAAAGCACCCAGATTCTCCACCACgagctcgaatttccccgatcgttcaaacatttggc
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gaattacgttaagcatgtaataattaacatgtaatgcatgacgttatttatgagatgggttttt
atgattagagtcccgcaattatacatttaatacgcgatagaaaacaaaatatagcgcgcaaact
aggataaattatcgcgcgcggtgtcatctatgttactagatcgggaattc

(Upper Case: RD29A Promoter; Underlined: Antisense GmFTB; Bold: Sense GmFTB; Lower case: NOS terminater)

SEQ ID NO:63

GAATTCAAATTTTTCGCCAGTTCTAAATATCCGGAAACCTCTTGGGATGCCATTGCCCATCTAT
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ACAGAAAGGCCATTGTTGAAGATACCTCTGCTGACATTGGTCCCCAAGTGGAAGCACCACCCCA
TGAGGAGCACCGTGGAGTAAGAAGACGTTCGAGCCACGTCGAAAAAGCAAGTGTGTTGATGTAG
TATCTCCATTGACGTAAGGGATGACGCACAATCCAACTATCCATCGCAAGACCATTGCTCTATA
TAAGAAAGTTAATATCATTTCGAGTGGCCACGCTGAGCTCGGATGGATTGGCTCCAGCAAATTA
GAGTACGGTCCAAGCACATGCTGAGGTAATGGGCACGAACCAGTATCAGTCATGGCACTGTACT
GGCTAACTGCGAGGCCACTGAGGCAGTAGCATGAATGATAGTGATCTCTGTTCTTTCCAGGCTT
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GTGTATTTACAGCAGCATAAGTCGTAGCTAGGTGAGGCAACTGTCCAGGTCCACCACTATATCC
ACCATCTTTATCCTGACATCGAGCTAAGAAGTCTATGATATCATTCTCAAGATCATCATCAAGT
GCTTCATCCAGCAAAGCAAGTGGATGAACCATCCAGTAGCATAGCCAAGGGCGATTGGCATCTA
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CCACAGCTCTAGCATGATGGATTTCGTGTTGGGCGCGGCCCCGAAGAGGGAGCGGTAGATGTCG
CCAACCCTGGCCTCCACCTTCATCTGCTCCACCTGCGTCACCGTGAGCCTCGGTAGGTCGGGAT
CCGCCgagctcgaatttccccgatcgttcaaacatttggcaataaagtttcttaagattgaatc
ctgttgccggtcttgcgatgattatcatataatttctgttgaattacgttaagcatgtaataat
taacatgtaatgcatgacgttatttatgagatgggtttttatgattagagtcccgcaattatac
atttaatacgcgatagaaaacaaaatatagcgcgcaaactaggataaattatcgcgcgcggtgt
catctatgttactagatcgggaattc

(Upper Case: MuA Promoter; Underlined: Antisense *Zea maize*-FTB; Lower case: NOS terminater)

SEQ ID NO:64

GAATTCAAATTTTTCGCCAGTTCTAAATATCCGGAAACCTCTTGGGATGCCATTGCCCATCTAT
CTGTAATTTATTGACGAAATAGACGAAAAGGAAGGTGGCTCCTATAAAGCACATCATTGCGATA
ACAGAAAGGCCATTGTTGAAGATACCTCTGCTGACATTGGTCCCCAAGTGGAAGCACCACCCCA
TGAGGAGCACCGTGGAGTAAGAAGACGTTCGAGCCACGTCGAAAAAGCAAGTGTGTTGATGTAG
TATCTCCATTGACGTAAGGGATGACGCACAATCCAACTATCCATCGCAAGACCATTGCTCTATA
TAAGAAAGTTAATATCATTTCGAGTGGCCACGCTGAGCTCGGATGGATTGGCTCCAGCAAATTA
GAGTACGGTCCAAGCACATGCTGAGGTAATGGGCACGAACCAGTATCAGTCATGGCACTGTACT
GGCTAACTGCGAGGCCACTGAGGCAGTAGCATGAATGATAGTGATCTCTGTTCTTTCCAGGCTT
ATCCCTCAAGCCTCCCTCTAGTACCTGAGAACAAAGTAGGATGTATTGTTGCAGGGCAATGTTA
TGGAAGAGTGGGCCAATTTGGTTGCTCTGTTGTATAAAATCAAATCCAAACTTCGCATAGTCCA
CAGCAGAGGAAGACTTATTCGCGGTGCACCCATATGAACTGGTGCTGCAGGCATCCTCTCCTGA
TGGCCTTTTGCAGGAATACGAGGACCTCAATTGCTTATCAACAATCGTAATTAACTTTTGTGTG
AAAGCAATGGCAGCTCCCTGCCAAAAGGAGTAGCAACCATCAACCAATTTATTAGTTCGTCCTT
GAAATCCGCATTCCACTCCTTGACGAAAAGCCACCCAGCCAATCAAACTAGGCAAGTCAACTTT
CTCTGCCTCATTAAGCAGGATCAAAGCAGCCAATCCACAGAATGTATACCCACCATGTGCTTCA
GCATAAGGCTCCCCAGCAATACCACCTTCATAAGTTTGACATCTTGCTATGTAGTCGCCTACAC
CTTTTGCCAGTTTAAAATCAAGAATATTCACAAGGCTGGCAACCGATATAGCGGTGTAGGAAGC
ACGGACATCAATTTCGCCACCATCATGCATTCTGAAAGCACCTGATACATCTTTCATCTGCAGC
ATAAAATTGTACAGGTTGCCCCTATTGATTGATGACAATGCTCTTTCGCTCCCTATTGTCACAA

GTGTATTTACAGCAGCATAAGTCGTAGCTAGGTGAGGCAACTGTCCAGGTCCACCACTATATCC
ACCATCTTTATCCTGACATCGAGCTAAGAAGTCTATGATATCATTCTCAAGATCATCATCAAGT
GCTTCATCCAGCAAAGCAAGTGGATGAACCATCCAGTAGCATAGCCAAGGGCGATTGGCATCTA
GAACATGAAAGGCTGGTCCCATATGCCTCAGCCCAGGCGTCAGATACTCGATATGCTGATCACG
CCACAGCTCTAGCATGATGGATTTCGTGTTGGGCGCGGCCCCGAAGAGGGAGCGGTAGATGTCG
CCAACCCTGGCCTCCACCTTCATCTGCTCCACCTGCGTCACCGTGAGCCTCGGTAGGTCGGGAT
CCGCCggatcc**GCTGGGGAGCCTTATGCTGAAGCACATGGTGGGTATACATTCTGTGGATTGGC**
TGCTTTGATCCTGCTTAATGAGGCAGAGAAAGTTGACTTGCCTAGTTTGATTGGCTGGGTGGCT
TTTCGTCAAGGAGTGGAATGCGGATTTCAAGGACGAACTAATAAATTGGTTGATGGTTGCTACT
CCTTTTGGCAGGGAGCTGCCATTGCTTTCACACAAAAGTTAATTACGATTGTTGATAAGCAATT
GAGGTCCTCGTATTCCTGCAAAAGGCCATCAGGAGAGGATGCCTGCAGCACCAGTTCATATGGG
TGCACCGCGAATAAGTCTTCCTCTGCTGTGGACTATGCGAAGTTTGGATTTGATTTTATACAAC
AGAGCAACCAAATTGGCCCACTCTTCCATAACATTGCCCTGCAACAATACATCCTACTTTGTTC
TCAGGTACTAGAGGGAGGCTTGAGGGATAAGCCTGGAAAGAACAGAGATCACTATCATTCATGC
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TACCTCAGCATGTGCTTGGACCGTACTCTAATTTGCTGGAGCCAATCCATCCaagcttgaattt
ccccgatcgttcaaacatttggcaataaagtttcttaagattgaatcctgttgccggtcttgcg
atgattatcatataatttctgttgaattacgttaagcatgtaataattaacatgtaatgcatga
cgttatttatgagatgggtttttatgattagagtcccgcaattatacatttaatacgcgataga
aaacaaaatatagcgcgcaaactaggataaattatcgcgcgcggtgtcatctatgttactagat
cggaagctt

(Upper Case: MuA Promoter; Underlined: Antisense *Zea maize*-FTB; Bold: Sense *Zea maize*-FTB; Lower case: NOS terminater)

Example 15: PCR Analysis of Putative Transgenic Plants

To verify that the putative transgenic plants carried the gene of interest PCR analysis was performed. Genomic DNA was isolated and PCR run according to standard protocols and conditions which are known to one of skill in the art. A typical reaction was performed in a volume of 25 µl and primer pairs used were dependent on the gene and promoter combination of the particular construct (Table 12).

Putative transgenic *Brassica napus* plants were screened using the primer combinations detailed in the table below. A representative gel showing PCR analysis results is shown in Figure 24 which represents transgenic plants carrying the pRD29A-anti-FTA construct. Transformants were confirmed in an analogous manner for each species and construct transformation done.

Table 12.

Construct Name	Primer Name	Primer Sequence (5'-3')
35S-antiFTA	SEQ ID NO:16	GCCGACAGTGGTCCCAAAGATGG
	SEQ ID NO:17	AAAGGATCCTCAAATTGCTGCCACTGTAAT
rd29A-antiFTA	SEQ ID NO:18	AAACCCGGGATGAATTTCGACGAGAACGTG
	SEQ ID NO:19	GCAAGACCGGCAACAGGA
rd29B-antiFTA	SEQ ID NO:20	TTTAAGCTTGACAGAAACAGTCAGCGAGAC

	SEQ ID NO:17	AAACCCGGGATGAATTTCGACGAGAACGTG
35S-DA-FTA	SEQ ID NO:21	GCTCTTCCTCCATGCCCA
	SEQ ID NO:19	GCAAGACCGGCAACAGGA
rd29A-DA-FTA	SEQ ID NO:22	TTTAAGCTTGGAGCCATAGATGCAATTCAA
	SEQ ID NO:23	CGGGCATTAGGAGGATGGGAA
35S-HP-FTB	SEQ ID NO:16	GCCGACAGTGGTCCCAAAGATGG
	SEQ ID NO:24	GTCCGGAATTCCCGGGTC
rd29A-HP-FTB	SEQ ID NO:22	TTTAAGCTTGGAGCCATAGATGCAATTCAA
	SEQ ID NO:24	GTCCGGAATTCCCGGGTC

Example 16: Southern Analysis

Genomic Southern analysis of anti-FTA transgenic *Arabidopsis thaliana*. The numbers indicate the line numbers. Five micrograms of genomic DNA of T1 plants was digested with *Hin*dIII (a unique site in the T-DNA plasmid) and separated in a 0.8% agarose gel. The NPTII coding region was used as the probe for radio-labeling. Figure 11 shows a typical result from Southern analysis indicating the presence of the transgene.

Example 17: Northern blots of antisense FTA lines

RNA was isolated from developing leaf tissue of five 35S-anti-FTA *Arabidopsis thaliana* lines (T3 plants). The blot was first probed with P^{32} labeled, single-stranded sense transcript of FTA (Figure 3 panel A) which detects antisense transcript, then stripped and re-probed with the single-stranded anti-sense transcript of FTA (Figure 12panel B) that detects the sense transcript. Figure 3 panel C shows the ethidium bromide stained gel for the blot. Approximately 5 μg of total RNA was loaded into each lane. Figure 3 indicates the accumulation of the transgene anti-sense transcript and a reduction in the sense transcript in transgenic plants.

Example 18: Western blot antisense FTA lines with Anti-FT-α antibodies.

The antibodies produced according to the methods of Example 27 were used to analyze protein extracts from transgenic plants on western blots. Lane 1 of Figure 13 is a molecular weight standard, lane 2 purified FTA protein, lanes 3-10 are protein extracts from the ERA1 mutant, wild type, and 4 lines of transgenic *Arabidopsis thaliana*. Figure 13 illustrates the reduction of detectable FTA protein in transgenic lines.

Example 19: ABA sensitivity of transgenic seedlings.

Seeds of wild type Columbia, era1-2 and T3 homozygous seeds of two antisense, drought tolerant lines of 35S-antisense-FTA were plated on minimum medium (1/2 MS) supplemented with no ABA (A), 0.3 μM (B), 0.5 μM (C) or 1.0 μM ABA (D). Plates were

chilled for 3 days in 4 ^{0}C in the dark, and incubated for 11 days at 22 ^{0}C with 24 hour continuous light. era1 and transgenic lines were more inhibited in germination than wild type plants. Results are shown in Figure 14.

Twelve day old seedling phenotypes of wild type Columbia, era1-2 and two drought tolerant 35S-antisense-FTA lines (9.9 & 21.2) in minimum medium without (A) or with (B) 1 μM ABA. Figure 15 shows the reduced root growth and development of era1 and transgenic lines relative to wild type plants. The 35S-antisense-FTA lines show reduced root growth, similar to the era1 mutant, in response to ABA.

A transgenic *Brassica napus* line carrying the 35S-antisense-FTA construct was assessed for ABA sensitivity. At about 10μm an effect was observed showing reduced seedling development and vigor at the cotyledon and first leaf stage, thereby indicating an increased sensitivity to ABA

ABA sensitivity is assessed in all transgenic plants engineered to have reduced or increased FTA or FTB expression or activity by the methods above. The ABA concentration used varies depending upon the species under examination.

Example 20: Drought Experiment

To assess the response of plants under water stress or drought one can expose plants to various situations. For example, the plant can be removed from soil or media and placed on paper towel for a period of time, such as 4 hours, then returned to a plate to continue growth and development. Survival and vigour can be assessed.

Alternatively one can impose a water stress in such a way as to more closely resemble a field situation by withholding water for a period of time, such as up to 6 days. Plants were grown five plants per four inch pot, in a replicated water-stress experiment. All pots were filled with equal amounts of homogeneous premixed and wetted soil. Growth conditions were 16 hour daylight (150-200 μmol/m^2/s) at 22 °C and 70% relative humidity. On the day that the first flower opened drought treatment was initiated first by equalizing the soil water content in each pot on a weight basis and then cessation of watering. At the end of the water stress treatment plants were typically either harvested for biomass data or re-watered to complete the life cycle and determination of biomass and yield data. Physiological parameters have been assessed under stressed and optimal conditions, for example, shoot and root biomass accumulation, soil water content, water loss alone or as a function of parameters such as biomass, seed yield, and leaf number and leaf area. Figure 16 shows photographs of wild type Columbia (A) and four 35S-antisense-FTA transgenic *Arabidopsis thaliana* lines (B,C,D,E) after 8 days of water stress

treatment. The control plant is visibly stressed and less healthy. This experiment has been conducted on transgenic lines containing vectors described by SEQ ID NO: 10, 46-64.

Drought or water stress tolerance is assessed in all transgenic plants engineered to have reduced or increased FTA or FTB expression or activity by the described methods.

Example 21: Analysis of Water Loss in Arabidopsis thaliana pRD29A-DA-FTA lines during drought stress

Plants were grown 5 plants per 4 inch pot and 6 pots per line. When the plants had grown to the first flower stage drought treatment was initiated as described in Example 20. Pots were weighed daily and at the end of the 7 day drought treatment all plants were harvested for shoot fresh weight and dry weight determinations. Figure 10 shows the water loss on a per shoot dry weight basis at 4 days of water stress treatment. Of the 31 lines examined in this experiment 25 showed lower water loss relative to the Columbia wild type, 22 of which were statistically significant. All lines had been assessed for ABA sensitivity as described in Example 14, increased ABA sensitivity (ABA^S) also correlated with a decreased water loss during drought treatment. Those lines determined to have wild type ABA sensitivity (ABA^{WT}) were the same 6 lines (lines 2, 36, 69, 29, 24, 21) that did not show a reduced water loss compared to wild type.

The above experiment was repeated using two ABA^S lines, one ABA^{WT} line and a Columbia control. Plants were harvested after 2,4 and 6 days of water stress treatment for shoot dry weight determinations. ABA^S transgenics had greater leaf and shoot biomass, greater soil water contents and lower water loss per shoot dry weight when compared to the ABA^{WT} or Columbia controls. Results were consistent at all three harvest stages.

The data shown in this example was obtained using transgenic plants carrying the pRD29A-DA-FTA construct. The experiment has also been conducted on lines carrying variations of this construct such as 35S-DA-FTA, pRD29A-antisense-FTA or 35S-antisense-FTA, with similar water stress tolerant trends observed. Soil water loss is assessed in all transgenic plants engineered to have reduced or increased FTA or FTB expression or activity by the described methods.

Example 22: Analysis of Shoot Fresh Weight in Arabidopsis thaliana pRD29A-DA-FTA lines during drought stress

Plants were grown 5 plants per 4 inch pot and 8 pots per line. When the plants had grown to the first flower stage drought treatment was initiated as described in Example 20. Plants were re-watered after 6 days drought treatment and allowed to recover for an additional 6 days. Plants were harvested and shoot fresh weights determined. Figure 20 shows the shoot fresh weights.

This experiment consisted of 25 transgenic lines, 2 of which are ABA^{WT} (line 2 and 69) and a Columbia wild type control. All 23 ABA^{S} transgenic lines had statistically significant greater shoot fresh weights, on average 44% greater.

The data shown in this example was obtained using transgenic plants carrying the pRD29A-DA-FTA construct. The experiment has been conducted on lines carrying variations of this construct such as 35S-DA-FTA, pRD29A-antisense-FTA or 35S-antisense-FTA, with similar trends observed.

Example 23: Analysis of seed yield in Arabidopsis thaliana pRD29A-DA-FTA lines during drought stress and under optimal conditions

Plants were grown 1 plant per 4 inch pot. When the plants had grown to the first flower stage drought treatment was initiated as described in Example 20. Plants were re-watered after 6 days drought treatment and allowed to grow to maturity. The optimal group was not exposed to the drought treatment.

Yield analysis indicates that although drought treatment results in decreased yields, the transgenics do not suffer as severely as controls and maintain a productivity advantage (Figure 21) as shown previously in Experiment 22. Comparison of the yields produced by the ABA^{S} transgenics versus the control plants show that a 15% greater yield was obtained under optimal conditions and a 20% increase under drought conditions. In the drought treatment group 8 of 9 transgenic lines showed greater yield than controls. Expression of yield of each line obtained under drought treatment as a percentage of its performance under optimum conditions indicates that 8 of 9 ABA^{S} lines outperformed the control line while 4 of 9 out performed the ABA^{WT} controls.

The data shown in this example was obtained using transgenic plants carrying the pRD29A-DA-FTA construct. The experiment has been conducted on lines carrying variations of this construct such as 35S-DA-FTA, pRD29A-antisense-FTA or 35S-antisense-FTA, with similar trends observed.

Example 24: Analysis of vegetative growth in Arabidopsis thaliana pRD29A-DA-FTA lines under optimum growth conditions

Plants were grown 1 plant per 3 inch pot and 8 pots per line. Plants were harvested at three stages and fresh weights determined. Vegetative stage was defined as 14 day old seedlings, bolting stage as the appearance of first flower (19-21 day seedlings) and mid-flowering as 6 days from first flower. At each of the above stages respectively 7, 8 and 10 of the 10 ABA^{S} transgenic lines tested showed statistically greater shoot fresh weight biomass than the control plants

(Figure 22). One Columbia line and an ABA^{WT} (line 2) line were used as the control group. Additionally, there was a statistically significant trend for the transgenic lines to have an increased number of rosette leaves.

The data shown in this example was obtained using transgenic plants carrying the pRD29A-DA-FTA construct. The experiment has been conducted on lines carrying variations of this construct such as 35S-DA-FTA, pRD29A-antisense-FTA or 35S-antisense-FTA, with similar trends observed.

Example 25: Analysis of Arabidopsis thaliana pRD29A-DA-FTA lines under drought treatment and biotic stress

Plants were grown 1 plant per 4 inch pot and 8 pots. When the plants had grown to the first flower stage drought treatment was initiated as described in Example 20. Plants were re-watered after 7 days drought treatment and allowed to grow to maturity. One Columbian control line (col) and one transgenic line were evaluated. Analysis of seed yield indicated less than normal yields, approximately 12% of expected optimal yield. It was determined that the soil used contained a fungal contaminant that was responsible for the reduced yields as the biotic stress could be negated by sterilization of the soil prior to use. This biotic stress was less severe in the transgenic line compared to the control which had a yield 22% of the transgenic line. In the drought treatment groups of plants the biotic stress was reduced however, transgenics outperformed controls by nearly 4.5 fold (Figure 23).

The data shown in this example was obtained using transgenic plants carrying the pRD29A-DA-FTA construct. The experiment has been conducted on lines carrying variations of this construct such as 35S-DA-FTA, pRD29A-antisense-FTA or 35S-antisense-FTA, with similar trends observed.

Example 26: Analysis of Arabidopsis thaliana pRD29A-DA-FTA lines for Stomatal number

The number of stomata on both the upper and lower surface of the leaf was assessed on two transgenic lines and a wild type Columbia control. Nail polish imprints were made of both upper and lower leaf surfaces of the fifth leaf, plants were at the early flowering stage. No differences in stoma density were observed.

The data shown in this example was obtained using transgenic plants carrying the pRD29A-DA-FTA construct. The experiment has been conducted on lines carrying variations of this construct such as 35S-DA-FTA, pRD29A-antisense-FTA or 35S-antisense-FTA, with similar trends observed.

Example 27: Production of polyclonal antibodies against FT-A and FT-B

The isolated *Arabidopsis thaliana* FT sequences were cloned into the *E. coli* expression vector derived from pET11D. To generate the Histidine tagged FT-B construct the *Arabidopsis thaliana* FT-B clone and pET vector were digested with *Bam*HI and ligated together. Restriction digests were performed to verify the orientation of the insert. To produce the FT-A construct the *Arabidopsis thaliana* FT-A clone and pET vector were digested with *Bam*HI and *Eco*RI and subsequently ligated together. The resultant plasmids directed the expression of fusion proteins containing 6 consecutive histidine residues at the N-termini of AtFTA and AtFTB. The fusion proteins were expressed in the bacterial host BL21(DE3) and purified using Hi-Trap chelating chromatography as described by the manufacturer (Pharmacia). The soluble fraction of the crude bacterial extract containing the His-FT fusion proteins were loaded to a Hi-Trap column (1.5 cm x 2.0 cm), and the proteins eluted with a 200 ml linear gradient of 0.0 to 0.3 M imidazole in column buffer (25 mM Tris-HCl, pH 7.5, 1 mM DTT). Fractions containing purified His-FT proteins were pooled, desalted and concentrated with a Centriprep-30 concentrator (Amicon). All purification steps were carried out at 4 °C. To generate an antibody, the purified fusion protein was further separated by SDS/PAGE and the Coomassie stained band corresponding to the fusion protein was excised. Protein was eluted from the gel slice by electroelution and then emulsified in Ribi adjuvant (Ribi Immunochem) to a final volume of 1 ml. His-AtFTA or His-AtFTB (250 μg) were injected into a 3 kg New Zealand rabbit on day 1 and booster injections given on day 21 and day 35 with 200 μg of the protein. High-titer antisera were obtained one week after the final injection. These antibodies were used in the western analysis of example 18, Figure 13.

Example 28: Screening for related genes

The transgenic plants of the invention can be used to identify genes which interact with the genes of the present invention. One can make use of the transgenic plants of the invention to screen for related genes, for example, suppressors, enhancers or modulators of gene expression or activity can be identified through genetic screening protocols. By way of example, a mutant library can be generated using the transgenic plants of the invention as the genetic background. Various methods are available and would be known to one of skill in the art. For example, chemical mutagens such as EMS can be used to induce point mutations in the genome, fast neutron irradiation of seeds can result in deletion mutations, T-DNA libraries can be produced that inactivate genes through insertional effects or activation tagging methods can be used to produce libraries with up-regulated genes. Analysis of these types of libraries can identify genes

which rescue or modulate the phenotypes observed in the transgenic plants of the present invention.

Example 29: RT-PCR amplification and cloning of CaaX prenyl proteases

Total RNA was isolated from leaf tissue of *Arabidopsis thaliana*, *Brassica napus* and *Glycine max*, using the Qiagen RNeasy kit and used as template to amplify the CPP genes by RT-PCR. Reaction conditions were as follows; 1X reaction buffer (10mM Tris-HCl pH 8.8, 1.5mM $MgCl_2$, 50mM KCl), dNTP's at 200μM, 1pM AtCPP BamFW and AtCPP SmaRV primers, 2.5U. Pfu DNA polymerase, and template plus water to a final volume of 100μL. Reactions were run at 1 minute 94°C, 1 minute 60°C, 1 minute 72°C, for 30 cycles. Primers used to PCR amplify *Arabidopsis* and *Brassica* sequences were those identified by SEQ ID NO:101 and SEQ ID NO:102. Primers used to PCR amplify the *Glycine* sequence were those identified by SEQ ID NO:149 and SEQ ID NO:150. PCR products were separated from the RT-PCR reaction mixture using the Qiagen PCR column spin kit and ligated into the prepared cloning vector, pBluescript KS+. The vector had been prepared by digestion with *Eco*RV and treated with *Taq* polymerase in the presence of dTTP to produce a 3' overhand suitable for ligation with the PCR products. The ligation products were transformed into *E. coli* DH5α cells, positive colonies selected and the resulting inserts sequenced. The above methodology is applicable to obtain homologous sequences and may require alternative primers.

Table 13.

AtCPP BamFW: (SEQ ID NO:101	5'-AAAGGATCCATGGCGATTCCTTTCATGG-3'
AtCPP SmaRV: ID NO:102)	5'-AAACCCGGGTTAATCTGTCTTCTTGTCTTCTCCA-3' (SEQ
GmCPP SmaFW: (SEQ ID NO:149)	5'-AAACCCGGGATGGCGTTTCCCTACATGGAAGCC -3'
GmCPP SacRV: ID NO:150)	5'-AAAGAGCTCTTAGTCTTCCTTCTTATCCGGTTCG -3' (SEQ

Example 30: Vector Construction

Construction of the pBI121-AtCPP construct (SEQ ID NO: 99) was prepared as follows. The pBI121 vector was digested with *Bam*HI and *Sma*I. The AtCPP, 1.4 kb DNA fragment from

RT-PCR (SEQ ID NO: 97) was digested with *Bam*HI and *Sma*I and ligated into the pBI121 vector. The GUS sequence was then removed by digestion with *Sma*I and *Eco*ICRI and the vector ligated after purification of the vector from the GUS insert to produce the pBI121-AtCPP vector (Figure 25A). This construct was used to further generate constructs expressing the CPP gene from *Brassica* and *Glycine*. To produce the pBI121-BnCPP construct (SEQ ID NO:142) primer pairs identified by SEQ ID NO:101 and SEQ ID NO:102 are used to PCR amplify the appropriate fragment which is ligated into the prepared parent vector. To produce the pBI121-GmCPP construct (SEQ ID NO:136) primer pairs identified by SEQ ID NO:149 and SEQ ID NO:150 are used to PCR amplify the appropriate fragment which is ligated into the prepared parent vector.

Construction of the pBI121-antisense-AtCPP construct (SEQ ID NO:130). The antisense fragment was produced using PCR amplification with SEQ ID NO:97 as template and primers identified as SEQ ID NO:106 and SEQ ID NO:107, listed in Table 14. This fragment was digested with *Bam*HI and *Sma*I and used to replace the sense fragment of the pBI121-AtCPP construct (SEQ ID NO:99), to yield SEQ ID NO:130 (Figure 25B) . This construct, SEQ ID NO:130, was used to further generate constructs expressing the antisense CPP gene from *Brassica* and *Glycine*. To produce the pBI121-antisense-BnCPP construct (SEQ ID NO:144) primer pairs identified by SEQ ID NO:151 and SEQ ID NO:152 are used to PCR amplify the appropriate fragment which is ligated into the prepared parent vector. To produce the pBI121-antisense-GmCPP construct (SEQ ID NO:138) primer pairs identified by SEQ ID NO:153 and SEQ ID NO:154 are used to PCR amplify the appropriate fragment which is ligated into the prepared parent vector.

Construction of the pBI121-HP-AtCPP construct (SEQ ID NO:100). The cloning strategy involved truncating the GUS gene of pBI121 and flanking the GUS sequence with a AtCPP fragment in the antisense orientation upstream of the GUS and in the sense orientation on the downstream side of GUS. The pBI121 vector was digested with *Sma*I and *Sac*I, the GUS sequence and the vector fragments were purified from one another. The isolated GUS fragment was digested using *Eco*RV and the 1079 bp. blunt ended *Eco*RV/*Sac*I fragment isolated. This was ligated back into the digested parent vector at the *Sma*I/*Sac*I sites. This intermediate vector was used in the subsequent production of the hair-pin vectors. The AtCPP fragment to be used as the gene specific hair-pin sequence was isolated by PCR. Primers identified as SEQ ID NO:103 and SEQ ID NO:104, listed in Table 14, were used to generate a 596 bp fragment. Cloning of the sense orientation fragment was achieved by digesting the PCR AtCPP fragment

with *Sac*I and ligation into the *Sac*I site at the 3' end of GUS. To insert the same fragment upsteam of GUS, the *Bam*HI site was opened and the ends blunted with Klenow. The PCR amplified AtCPP fragment was digested with *Eco*lCRI, which is an isoschizomer of *Sac*I but leaves blunt ends, and ligated into the blunted *Bam*HI site of the vector to yield the final construct (Figure 25C). The intermediate construct used to produce SEQ ID NO:100 above contained only the truncated GUS gene and no CPP sequences this intermediate vector was used to further generate constructs expressing hair-pin CPP gene constructs from *Brassica* and *Glycine*. To produce the pBI121-HP-BnCPP construct (SEQ ID NO:143) primer pairs identified by SEQ ID NO:153 and SEQ ID NO:154 are used to PCR amplify the sense fragment and primer pairs identified by SEQ ID NO:155 and SEQ ID NO:156 are used to PCR amplify the antisense fragment. These fragments are cloned into the prepared intermediate vector described above. To produce the pBI121-HP-GmCPP construct (SEQ ID NO:137) primer pairs identified by SEQ ID NO:157 and SEQ ID NO:158 are used to PCR amplify the sense fragment and primer pairs identified by SEQ ID NO:159 and SEQ ID NO:160 are used to PCR amplify the antisense fragment. These fragments are cloned into the prepared intermediate vector described above.

The above vector constructs were modified to place the genes under the control of alternative promoters, such as, but not limited to, the RD29A or MuA . This was accomplished by excising the 35S promoter sequence and replacing it with an appropriate promoter sequence. In this way SEQ ID NO's:134 and 135 were generated and SEQ ID NO's:133, 136-148 can be constructed.

Table 14

AtCPP-HP-SacFW 5'-CTGGAGCTCTTTTACCGAGGTTGGGCCTTGATCC-3' (SEQ ID NO:103)

AtCPP-HP-SacRV 5'-ATTGAGCTCCCAATGTCCAAGCTCGTGTGCAATA-3' (SEQ ID NO:104)

AtCPP-anti-SmaFW 5'-AAACCCGGGATGGCGATTCCTTTCATGG-3' (SEQ ID NO:106)

AtCPP-anti-BamRV 5'-AAAGGATCCTTAATCTGTCTTCTTGTCTTCTCCA-3' (SEQ ID NO:107)

BnCPP-anti-SmaFW 5'-AAACCCGGGATGGCGATTCCTTTCATGG -3' (SEQ ID NO:151)

BnCPP-anti-BamRV 5'-AAAGGATCCTTAATCTGTCTTCTTGTCTTCTCC -3' (SEQ ID NO:152)

BnCPP-HP-Sac-FW 5'- AAAGAGCTCTTCTACCAATGGTGGGACTCG -3' (SEQ ID NO:153)

BnCPP-HP-Sac-RV 5'- AAAGAGCTCCCAGTGTCCCAGCTCGTGTG -3' (SEQ ID NO:154)

BnCPP-HP-BamFW 5'- AAAGGATCCTTCTACCAATGGTGGGACTCG -3' (SEQ ID NO:155)

BnCPP-HP-XbaRV 5'- AAATCTAGACCAGTGTCCCAGCTCGTGTG -3' (SEQ ID NO:156)

GmCPP-HP-Sac-FW
5'-GATGAGCTCACAAGATCAAGTCACAGCAATGCCT -3' (SEQ ID NO:157)

GmCPP-HP-Sac-RV 5'- AAAGAGCTCCCGGTTCGTCCAGCGCGGCC -3' (SEQ ID NO:158)

GmCPP-HP-BamFW
5'- GATGGATCCACAAGATCAAGTCACAGCAATGCCT -3' (SEQ ID NO:159)

GmCPP-HP-XbaRV 5'- CCTTCTAGACCGGTTCGTCCAGCGCGGCC -3' (SEQ ID NO:160)

Example 31: Sequence Analysis

***Arabidopsis thaliana* CPP (AtCPP)**

A disclosed nucleic acid of 1275 nucleotides (SEQ ID NO:97) and also referred to as AtCPP, is shown in Table 15.

Table 15A. AtCPP Nucleotide Sequence (SEQ ID NO:97).

ATGGCGATTCCTTTCATGGAAACCGTCGTGGGTTTTATGATAGTGATGTACATTTTTGAG
ACGTATTTGGATCTGAGGCAACTCACTGCTCTCAAGCTTCCAACTCTCCCGAAAACCTTG
GTTGGTGTAATTAGCCAAGAGAAGTTTGAGAAATCACGAGCATACAGTCTTGACAAAAGC
TATTTTCACTTTGTTCATGAGTTTGTAACTATACTTATGGACTCTGCAATTTTGTTCTTT
GGGATCTTGCCTTGGTTTTGGAAGATGTCTGGAGCTGTTTTACCGAGGTTGGGCCTTGAT
CCGGAGAATGAAATACTGCATACTCTTTCATTCTTGGCTGGTGTTATGACATGGTCACAG
ATCACTGATTTGCCATTTTCTTTGTACTCAACTTTCGTGATCGAGTCTCGGCATGGGTTC
AACAAACAAACAATATGGATGTTCATTAGGGACATGATCAAAGGAACATTCCTCTCTGTC
ATACTAGGCCCACCCATTGTTGCTGCGATAATTTTCATAGTCCAGAAAGGAGGTCCTTAT
CTTGCCATCTATCTGTGGGCATTCATGTTTATCCTGTCTCTAGTGATGATGACTATATAC
CCGGTCTTGATAGCACCGCTCTTCAACAAATTCACTCCTCTTCCAGATGGAGACCTCCGG
GAGAAGATTGAGAAACTTGCTTCTTCCCTAAAGTTTCCTTTGAAGAAGCTGTTTGTTGTC
GATGGATCTACAAGGTCAAGCCATAGCAATGCTTACATGTATGGTTTCTTTAAGAACAAA
AGGATTGTTCTTTATGATACGTTGATTCAGCAGTGCAAGAATGAGGATGAAATTGTGGCG
GTTATTGCACACGAGCTTGGACATTGGAAACTGAATCACACTACATACTCGTTCATTGCA
GTTCAAATCCTTGCCTTCTTACAATTTGGAGGATACACTCTTCTCAGAAACTCCACTGAT
CTCTTCAGGAGTTTCGGATTTGATACACAGCCTGTTCTCATTGGTTTGATCATATTTCAG
CACACTGTAATACCACTGCAACATCTAGTAAGCTTTGGCCTGAACCTCGTTAGTCGAGCG
TTTGAGTTTCAGGCTGATGCTTTTGCTGTGAAGCTTGACTATGCAAAAGATCTTCGTCCT

GCTCTAGTGAAACTACAGGAAGAGAACTTATCAACAATGAACACTGATCCATTGTACTCA
GCTTATCACTACTCACATCCTCCTCTTGTTGAAAGGCTTCGAGCCACTGATGGAGAAGAC
AAGAAGACAGATTAA

A disclosed CPP polypeptide (SEQ ID NO:98) encoded by SEQ ID NO:97 has 424 amino acid residues and is presented in Table 15B using the one-letter amino acid code.

Table 15B. Encoded CPP protein sequence (SEQ ID NO:98).

MAIPFMETVVGFMIVMYIFETYLDLRQLTALKLPTLPKTLVGVISQEKFEKSRAYSLDKS
YFHFVHEFVTILMDSAILFFGILPWFWKMSGAVLPRLGLDPENEILHTLSFLAGVMTWSQ
ITDLPFSLYSTFVIESRHGFNKQTIWMFIRDMIKGTFLSVILGPPIVAAIIFIVQKGGPY
LAIYLWAFMFILSLVMMTIYPVLIAPLFNKFTPLPDGDLREKIEKLASSLKFPLKKLFVV
DGSTRSSHSNAYMYGFFKNKRIVLYDTLIQQCKNEDEIVAVIAHELGHWKLNHTTYSFIA
VQILAFLQFGGYTLLRNSTDLFRSFGFDTQPVLIGLIIFQHTVIPLQHLVSFGLNLVSRA
FEFQADAFAVKLDYAKDLRPALVKLQEENLSTMNTDPLYSAYHYSHPPLVERLRATDGED
KKTD

The present invention also includes a nucleic acid sequence complimentary to the *Arabidopsis thaliana* CaaX prenyl protease of SEQ ID NO:97. The disclosed complimentary sequence is shown as SEQ ID NO:115.

SEQ ID NO:115

TTAATCTGTCTTCTTGTCTTCTCCATCAGTGGCTCGAAGCCTTTCAACAAGAGGAGGATGTGAG
TAGTGATAAGCTGAGTACAATGGATCAGTGTTCATTGTTGATAAGTTCTCTTCCTGTAGTTTCA
CTAGAGCAGGACGAAGATCTTTTGCATAGTCAAGCTTCACAGCAAAAGCATCAGCCTGAAACTC
AAACGCTCGACTAACGAGGTTCAGGCCAAAGCTTACTAGATGTTGCAGTGGTATTACAGTGTGC
TGAAATATGATCAAACCAATGAGAACAGGCTGTGTATCAAATCCGAAACTCCTGAAGAGATCAG
TGGAGTTTCTGAGAAGAGTGTATCCTCCAAATTGTAAGAAGGCAAGGATTTGAACTGCAATGAA
CGAGTATGTAGTGTGATTCAGTTTCCAATGTCCAAGCTCGTGTGCAATAACCGCCACAATTTCA
TCCTCATTCTTGCACTGCTGAATCAACGTATCATAAAGAACAATCCTTTTGTTCTTAAAGAAAC
CATACATGTAAGCATTGCTATGGCTTGACCTTGTAGATCCATCGACAACAAACAGCTTCTTCAA
AGGAAACTTTAGGGAAGAAGCAAGTTTCTCAATCTTCTCCCGGAGGTCTCCATCTGGAAGAGGA
GTGAATTTGTTGAAGAGCGGTGCTATCAAGACCGGGTATATAGTCATCATCACTAGAGACAGGA
TAAACATGAATGCCCACAGATAGATGGCAAGATAAGGACCTCCTTTCTGGACTATGAAAATTAT
CGCAGCAACAATGGGTGGGCCTAGTATGACAGAGAGGAATGTTCCTTTGATCATGTCCCTAATG
AACATCCATATTGTTTGTTTGTTGAACCCATGCCGAGACTCGATCACGAAAGTTGAGTACAAAG

AAAATGGCAAATCAGTGATCTGTGACCATGTCATAACACCAGCCAAGAATGAAAGAGTATGCAG
TATTTCATTCTCCGGATCAAGGCCCAACCTCGGTAAAACAGCTCCAGACATCTTCCAAAACCAA
GGCAAGATCCCAAAGAACAAAATTGCAGAGTCCATAAGTATAGTTACAAACTCATGAACAAAGT
GAAAATAGCTTTTGTCAAGACTGTATGCTCGTGATTTCTCAAACTTCTCTTGGCTAATTACACC
AACCAAGGTTTTCGGGAGAGTTGGAAGCTTGAGAGCAGTGAGTTGCCTCAGATCCAAATACGTC
TCAAAAATGTACATCACTATCATAAAACCCACGACGGTTTCCATGAAAGGAATCGCCAT

Due to the nature of the cloning strategy the sequence presented is not full length but is missing the 5' and 3' non-translated regions. The percent identities of the *Arabidopsis thaliana* nucleotide sequence and its encoded amino acid sequence to that of other CPP sequences as determined by ClustalW analysis are shown in Figure 26.

Using the sequences disclosed herein as hybridization probes, one is able to screen and isolate full length sequences from cDNA or genomic libraries or use the rapid amplification of cDNA ends (RACE) technology or other such PCR techniques.

***Brassica napus* CPP (BnCPP)**

A disclosed nucleic acid of 1275 nucleotides (SEQ ID NO:109) and also referred to as BnCPP, is shown in Table 16.

Table 16A. BnCPP Nucleotide Sequence (SEQ ID NO:109).

ATGGCGATTCCTTTCATGGAAACCGTCGTTGGTTTTATGATAGTGATGTACGTT
TTTGAGACGTATTTGGATCTGAGGCAACATACTGCTCTCAAGCTTCCCACTCTC
CCAAAGACTTTGGTTGGAGTCATTAGCCAAGAGAAGTTTGAGAAATCTCGAGCT
TACAGTCTTGACAAAAGCCATTTTCACTTTGTTCATGAGTTTGTTACTATACTT
ATGGACTCTGCGATTCTGTTCTTTGGGATCTTGCCTTGGTTTTGGAAGATATCT
GGCGGCTTTCTACCAATGGTGGGACTCGATCCAGAGAATGAAATCCTGCACACT
CTTTCATTCTTGGCTGGTCTTATGACATGGTCACAGATCACTGATTTGCCATTT
TCTTTGTACTCAACTTTCGTGATCGAGTCTCGGCATGGGTTCAACAAACAAACA
ATATGGATGTTCATTAGGGACATGATCAAAGGAATACTCCTCTCTGTCATACCT
GCCCCTCCTATCGTTGCCGCAATTATTGTTATAGTTCAGAAAGGAGGTCCTTAC
CTCGCCATCTATCTGTGGGCATTCATGTTTATCCTGTCTCTAGTGATGATGACT
ATATACCCTGTTTTGATTGCACCTCTTTTCAACAAGTTCACTCCTCTTCCTGAT
GGAGACCTCCGGGAGAAGATTGAGAAACTTGCTTCTTCTCTAAAGTTTCCTCTG

AAGAAGCTGTTTGTTGTCGATGGATCTACAAGGTCAAGCCATAGTAATGCTTAC
ATGTATGGTTTCTTCAAGAACAAAAGGATTGTTCTTTATGACACATTGATTCAG
CAGTGCCAGAATGAGAATGAAATTGTGGCGGTTATTGCACACGAGCTGGGACAC
TGGAAGCTGAATCACACTACATACTCGTTCATTGCTGTTCAAATCCTTGCCTTC
TTGCAATTTGGAGGATACACTCTTGTCAGAAACTCCACTGATCTCTTCAGGAGT
TTTGGTTTTGATACACAACCAGTTCTCATTGGTTTGATCATATTTCAGCACACT
GTAATACCACTTCAACACCTAGTAAGCTTTGACCTCAACCTTGTTAGTCGAGCG
TTTGAGTTTCAGGCTGATGCTTTTGCAGTGAATCTTGGTTATGCAAAGGATCTA
CGTCCTGCCCTAGTGAAGCTACAGGAAGAGAACTTATCAGCGATGAACACAGAC
CCATTGTACTCAGCTTATCACTACTCACACCCTCCTCTTGTAGAGAGGCTTCGA
GCCATTGATGGAGAAGACAAGAAGACAGATTAA

A disclosed CPP polypeptide (SEQ ID NO:110) encoded by SEQ ID NO:109 has 424 amino acid residues and is presented in Table 16B using the one-letter amino acid code.

Table 16B. Encoded CPP protein sequence (SEQ ID NO:110).

MAIPFMETVVGFMIVMYVFETYLDLRQHTALKLPTLPKTLVGVISQEKFEKSRA
YSLDKSHFHFVHEFVTILMDSAILFFGILPWFWKISGGFLPMVGLDPENEILHT
LSFLAGLMTWSQITDLPFSLYSTFVIESRHGFNKQTIWMFIRDMIKGILLSVIP
APPIVAAIIVIVQKGGPYLAIYLWAFMFILSLVMMTIYPVLIAPLFNKFTPLPD
GDLREKIEKLASSLKFPLKKLFVVDGSTRSSHSNAYMYGFFKNKRIVLYDTLIQ
QCQNENEIVAVIAHELGHWKLNHTTYSFIAVQILAFLQFGGYTLVRNSTDLFRS
FGFDTQPVLIGLIIFQHTVIPLQHLVSFDLNLVSRAFEFQADAFAVNLGYAKDL
RPALVKLQEENLSAMNTDPLYSAYHYSHPPLVERLRAIDGEDKKTD

The present invention also includes a nucleic acid sequence complimentary to the *Brassica napus* CaaX prenyl protease of SEQ ID NO:109. The disclosed complimentary sequence is shown as SEQ ID NO:111.

SEQ ID NO:111

TTAATCTGTCTTCTTGTCTTCTCCATCAATGGCTCGAAGCCTCTCTACAAGAGGAGGGTGTGAG
TAGTGATAAGCTGAGTACAATGGGTCTGTGTTCATCGCTGATAAGTTCTCTTCCTGTAGCTTCA
CTAGGGCAGGACGTAGATCCTTTGCATAACCAAGATTCACTGCAAAAGCATCAGCCTGAAACTC
AAACGCTCGACTAACAAGGTTGAGGTCAAAGCTTACTAGGTGTTGAAGTGGTATTACAGTGTGC
TGAAATATGATCAAACCAATGAGAACTGGTTGTGTATCAAAACCAAAACTCCTGAAGAGATCAG

TGGAGTTTCTGACAAGAGTGTATCCTCCAAATTGCAAGAAGGCAAGGATTTGAACAGCAATGAA
CGAGTATGTAGTGTGATTCAGCTTCCAGTGTCCCAGCTCGTGTGCAATAACCGCCACAATTTCA
TTCTCATTCTGGCACTGCTGAATCAATGTGTCATAAAGAACAATCCTTTTGTTCTTGAAGAAAC
CATACATGTAAGCATTACTATGGCTTGACCTTGTAGATCCATCGACAACAAACAGCTTCTTCAG
AGGAAACTTTAGAGAAGAAGCAAGTTTCTCAATCTTCTCCCGGAGGTCTCCATCAGGAAGAGGA
GTGAACTTGTTGAAAAGAGGTGCAATCAAAACAGGGTATATAGTCATCATCACTAGAGACAGGA
TAAACATGAATGCCCACAGATAGATGGCGAGGTAAGGACCTCCTTTCTGAACTATAACAATAAT
TGCGGCAACGATAGGAGGGGCAGGTATGACAGAGAGGAGTATTCCTTTGATCATGTCCCTAATG
AACATCCATATTGTTTGTTTGTTGAACCCATGCCGAGACTCGATCACGAAAGTTGAGTACAAAG
AAAATGGCAAATCAGTGATCTGTGACCATGTCATAAGACCAGCCAAGAATGAAAGAGTGTGCAG
GATTTCATTCTCTGGATCGAGTCCCACCATTGGTAGAAAGCCGCCAGATATCTTCCAAAACCAA
GGCAAGATCCCAAAGAACAGAATCGCAGAGTCCATAAGTATAGTAACAAACTCATGAACAAAGT
GAAAATGGCTTTTGTCAAGACTGTAAGCTCGAGATTTCTCAAACTTCTCTTGGCTAATGACTCC
AACCAAAGTCTTTGGGAGAGTGGGAAGCTTGAGAGCAGTATGTTGCCTCAGATCCAAATACGTC
TCAAAAACGTACATCACTATCATAAAACCAACGACGGTTTCCATGAAAGGAATCGCCAT

Due to the nature of the cloning strategy the sequence presented is not full length but is missing the 5' and 3' non-translated regions. The percent identities of the *Brassica napus* nucleotide sequence and its encoded amino acid sequence to that of other CPP sequences as determined by ClustalW analysis are shown in Figure 26.

Using the sequences disclosed herein as hybridization probes, one is able to screen and isolate full length sequences from cDNA or genomic libraries or use the rapid amplification of cDNA ends (RACE) technology or other such PCR techniques.

***Glycine max* CPP (GmCPP)**

A disclosed nucleic acid of 1275 nucleotides (SEQ ID NO:112) and also referred to as GmCPP, is shown in Table 17.

Table 17A. GmCPP Nucleotide Sequence (SEQ ID NO:112).

ATGGCGTTTCCCTACATGGAAGCCGTTGTCGGATTTATGATATTAATGTACATT
TTTGAAACTTACTTGGATGTGCGACAACATAGGGCCCTCAAACTTCCTACTCTT
CCAAAGACTTTAGAGGGTGTTATCAGCCAAGAGAAATTTGAGAAATCTAGAGCC
TATAGTCTTGATAAAAGCCACTTCCATTTTGTTCACGAGTTTGTGACAATAGTG

ACAGACTCTACAATTTTGTACTTTGGGGTATTGCCCTGGTTTTGGAAGAAATCA
GGAGATTTTATGACAATAGCTGGTTTCAATGCTGAGAATGAAATACTGCATACC
CTTGCCTTCTTAGCAGGGCTGATGATTTGGTCACAGATAACAGATTTGCCCTTT
TCTCTGTACTCAACTTTTGTGATTGAGGCCCGTCATGGTTTTAATAAGCAAACA
CCATGGTTATTCTTTAGGGACATGCTTAAAGGAATTTTCCTTTCTGTAATAATT
GGTCCACCTATTGTGGCTGCAATCATTGTAATAGTACAGAAAGGAGGTCCATAC
TTGGCCATCTATCTTTGGGTTTTTACGTTTGGTCTTTCTATTGTGATGATGACC
CTTTATCCAGTACTAATAGCTCCACTCTTCAATAAGTTCACTCCACTTCCAGAT
GGTCAACTCAGGGAGAAAATCGAGAAACTTGCTTCCTCCCTCAACTATCCGTTA
AAGAAACTATTTGTTGTCGATGGATCCACAAGATCAAGTCACAGCAATGCCTAT
ATGTATGGATTCTTCAAGAACAAGAGGATTGTCCCTTATGACACATTAATTCAA
CAGTGCAAAGACGATGAGGAAATTGTTGCTGTTATTGCCCATGAGTTGGGACAC
TGGAAGCTCAACCATACTGTGTACACATTTGTTGCTATGCAGATTCTTACACTT
CTACAATTTGGAGGATATACACTAGTGCGAAATTCAGCTGATCTGTATCGAAGC
TTTGGGTTTGATACGCAGCCAGTCCTCATTGGGCTCATCATATTTCAGCATACT
GTAATCCCACTTCAGCAATTGGTCAGCTTTGGTCTGAACCTAGTCAGCCGATCA
TTTGAATTTCAGGCTGATGGCTTTGCCAAGAAGCTTGGATATGCATCTGGATTA
CGCGGTGGTCTTGTGAAACTACAGGAGGAGAATCTGTCAGCTATGAATACAGAT
CCTTGGTACTCTGCTTATCACTATTCTCATCCTCCCCTTGTTGAAAGATTGGCC
GCGCTGGACGAACCGGATAAGAAGGAAGACTAA

A disclosed CPP polypeptide (SEQ ID NO:113) encoded by SEQ ID NO:112 has 424 amino acid residues and is presented in Table 17B using the one-letter amino acid code.

Table 17B. Encoded CPP protein sequence (SEQ ID NO:113).

MAFPYMEAVVGFMILMYIFETYLDVRQHRALKLPTLPKTLEGVISQEKFEKSRAYSLDKS
HFHFVHEFVTIVTDSTILYFGVLPWFWKKSGDFMTIAGFNAENEILHTLAFLAGLMIWSQ
ITDLPFSLYSTFVIEARHGFNKQTPWLFFRDMLKGIFLSVIIGPPIVAAIIVIVQKGGPY
LAIYLWVFTFGLSIVMMTLYPVLIAPLFNKFTPLPDGQLREKIEKLASSLNYPLKKLFVV
DGSTRSSHSNAYMYGFFKNKRIVPYDTLIQQCKDDEEIVAVIAHELGHWKLNHTVYTFVA
MQILTLLQFGGYTLVRNSADLYRSFGFDTQPVLIGLIIFQHTVIPLQQLVSFGLNLVSRS
FEFQADGFAKKLGYASGLRGGLVKLQEENLSAMNTDPWYSAYHYSHPPLVERLAALDEPD
KKED

The present invention also includes a nucleic acid sequence complimentary to the *Glycine max* CaaX prenyl protease of SEQ ID NO:112. The disclosed complimentary sequence is shown as SEQ ID NO:114.

SEQ ID NO:114

TTAGTCTTCCTTCTTATCCGGTTCGTCCAGCGCGGCCAATCTTTCAACAAGGGGAGGATGAGAA
TAGTGATAAGCAGAGTACCAAGGATCTGTATTCATAGCTGACAGATTCTCCTCCTGTAGTTTCA
CAAGACCACCGCGTAATCCAGATGCATATCCAAGCTTCTTGGCAAAGCCATCAGCCTGAAATTC
AAATGATCGGCTGACTAGGTTCAGACCAAAGCTGACCAATTGCTGAAGTGGGATTACAGTATGC
TGAAATATGATGAGCCCAATGAGGACTGGCTGCGTATCAAACCCAAAGCTTCGATACAGATCAG
CTGAATTTCGCACTAGTGTATATCCTCCAAATTGTAGAAGTGTAAGAATCTGCATAGCAACAAA
TGTGTACACAGTATGGTTGAGCTTCCAGTGTCCCAACTCATGGGCAATAACAGCAACAATTTCC
TCATCGTCTTTGCACTGTTGAATTAATGTGTCATAAGGGACAATCCTCTTGTTCTTGAAGAATC
CATACATATAGGCATTGCTGTGACTTGATCTTGTGGATCCATCGACAACAAATAGTTTCTTTAA
CGGATAGTTGAGGGAGGAAGCAAGTTTCTCGATTTTCTCCCTGAGTTGACCATCTGGAAGTGGA
GTGAACTTATTGAAGAGTGGAGCTATTAGTACTGGATAAAGGGTCATCATCACAATAGAAAGAC
CAAACGTAAAAACCCAAAGATAGATGGCCAAGTATGGACCTCCTTTCTGTACTATTACAATGAT
TGCAGCCACAATAGGTGGACCAATTATTACAGAAAGGAAAATTCCTTTAAGCATGTCCCTAAAG
AATAACCATGGTGTTTGCTTATTAAAACCATGACGGGCCTCAATCACAAAAGTTGAGTACAGAG
AAAAGGGCAAATCTGTTATCTGTGACCAAATCATCAGCCCTGCTAAGAAGGCAAGGGTATGCAG
TATTTCATTCTCAGCATTGAAACCAGCTATTGTCATAAAATCTCCTGATTTCTTCCAAAACCAG
GGCAATACCCCAAAGTACAAAATTGTAGAGTCTGTCACTATTGTCACAAACTCGTGAACAAAAT
GGAAGTGGCTTTTATCAAGACTATAGGCTCTAGATTTCTCAAATTTCTCTTGGCTGATAACACC
CTCTAAAGTCTTTGGAAGAGTAGGAAGTTTGAGGGCCCTATGTTGTCGCACATCCAAGTAAGTT
TCAAAAATGTACATTAATATCATAAATCCGACAACGGCTTCCATGTAGGGAAACGCCAT

Due to the nature of the cloning strategy the sequence presented is not full length but is missing the 5' and 3' non-translated regions. The percent identities of the *Glycine max* nucleotide sequence and its encoded amino acid sequence to that of other CPP sequences as determined by ClustalW analysis are shown in Figure 26.

Using the sequences disclosed herein as hybridization probes, one is able to screen and isolate full length sequences from cDNA or genomic libraries or use the rapid amplification of cDNA ends (RACE) technology or other such PCR techniques.

The CPP nucleic acids and amino acids disclosed above have homology to other disclosed CPP sequences (GenBank ID NOs: AL161491 (AT4g01320), AF007269 and AF353722; WO 02/16625 A2). The homology between these and other sequences is shown in the ClustalW alignment analysis shown in Tables 18A-18B.

Table 18A. ClustalW Nucleic Acid Analysis of CaaX Prenyl Protease

1: PPI-AtCPP SEQ ID NO:97
2: PPI-BnCPP SEQ ID NO:109
3: PPI-GmCPP SEQ ID NO:112
4: BASF_AT1 SEQ ID NO:116
5: BASF_AT2 SEQ ID NO:118
6: BASF-Corn SEQ ID NO:120
7: BASF-Gm SEQ ID NO:122
8: AFC1 SEQ ID NO:124
9: AT4g01320 SEQ ID NO:126
10: AF007269 SEQ ID NO:128

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CLUSTAL W (1.81) multiple sequence alignment


PPI-GmCPP       ------------------------------------------------------------
BASF-Gm         ------------------------------------------------------------
AT4g01320       ------------------------------------------------------------
AF007269        ATGGCGATTCCTTTCATGGAAACCGTCGTGGGTAAGCTTCAAAACCTTTTTCTGAGACAT
PPI-AtCPP       ------------------------------------------------------------
BASF_AT2        ------------------------------------------------------------
afc1            ------------------------------------------------------------
BASF_AT1        ------------------------------------------------------------
PPI-BnCPP       ------------------------------------------------------------
BASF-Corn       ------------------------------------------------------------


PPI-GmCPP       ------------------------------------------------------------
BASF-Gm         ------------------------------------------------------------
AT4g01320       ------------------------------------------------------------
AF007269        TTTACTATCCTGTTTCACTCATCGTATTTCGTTTTTGTTTGGGTTTTGCTTTCTGTGTTG
PPI-AtCPP       ------------------------------------------------------------
BASF_AT2        ------------------------------------------------------------
afc1            ------------------------------------------------------------
BASF_AT1        ------------------------------------------------------------
PPI-BnCPP       ------------------------------------------------------------
BASF-Corn       ------------------------------------------------------------


PPI-GmCPP       ------------------------------------------------------------
BASF-Gm         ------------------------------------------------------------
AT4g01320       ------------------------------------------------------------
AF007269        TGTGTGTTGAGATTCCATGACTCGTTTGTTTCATATACCATCGTCTCTGCTTCTCGTTTC
PPI-AtCPP       ------------------------------------------------------------
BASF_AT2        ------------------------------------------------------------
afc1            ------------------------------------------------------------
BASF_AT1        ------------------------------------------------------------
PPI-BnCPP       ------------------------------------------------------------
BASF-Corn       ------------------------------------------------------------


PPI-GmCPP       ------------------------------------------------------------
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BASF-Gm         ------------------------------------------------------------
AT4g01320       ------------------------------------------------------------
AF007269        TAAATTTTGTTCTTTTCTAATAGTGCGTACCTTGATCTGAGGTTTTATTACTCCTACTAG
PPI-AtCPP       ------------------------------------------------------------
BASF_AT2        ------------------------------------------------------------
afc1            ------------------------------------------------------------
BASF_AT1        ------------------------------------------------------------
PPI-BnCPP       ------------------------------------------------------------
BASF-Corn       ------------------------------------------------------------


PPI-GmCPP       ------------------------------------------------------------
BASF-Gm         ------------------------------------------------------------
AT4g01320       ------------------------------------------------------------
AF007269        TTTCTTGTCTTACTCGTGCGTTTGATTTGATTTGAGCTTATGTGATTTCATCATCTCTTC
PPI-AtCPP       ------------------------------------------------------------
BASF_AT2        ------------------------------------------------------------
afc1            ------------------------------------------------------------
BASF_AT1        ------------------------------------------------------------
PPI-BnCPP       ------------------------------------------------------------
BASF-Corn       ------------------------------------------------------------


PPI-GmCPP       ------------------------------------------------------------
BASF-Gm         ------------------------------------------------------------
AT4g01320       ------------------------------------------------------------
AF007269        CTCGGTTTTAGAATGTACGGAGCTTCTCTGTTAACCAAAATCTAGGATTTGGGAAGAAAA
PPI-AtCPP       ------------------------------------------------------------
BASF_AT2        ------------------------------------------------------------
afc1            ------------------------------------------------------------
BASF_AT1        ------------------------------------------------------------
PPI-BnCPP       ------------------------------------------------------------
BASF-Corn       ------------------------------------------------------------


PPI-GmCPP       ------------------------------------------------------------
BASF-Gm         ------------------------------------------------------------
AT4g01320       ------------------------------------------------------------
AF007269        GTCGGAGTCTTTTTTTTCCTCATTCCCGATTGGAAATTGAGAATCTTGAAATTTTTCTTT
PPI-AtCPP       ------------------------------------------------------------
BASF_AT2        ------------------------------------------------------------
afc1            ------------------------------------------------------------
BASF_AT1        ------------------------------------------------------------
PPI-BnCPP       ------------------------------------------------------------
BASF-Corn       ------------------------------------------------------------


PPI-GmCPP       ------------------------------------------------------------
BASF-Gm         ------------------------------------CTAATACGACTCACTATAGGGC
AT4g01320       ------------------------------------------------------------
AF007269        GTTCAAGTCATACAGCTTGAGGTTTTGGGTTTTCTTGTCAGGGTATTATTATGTTCGTGA
PPI-AtCPP       ------------------------------------------------------------
BASF_AT2        ------------------------------------------------------------
afc1            ------------------------------------------------------------
BASF_AT1        ------------------------------------------------------------
PPI-BnCPP       ------------------------------------------------------------
BASF-Corn       ------------------------------------------------------------

PPI-GmCPP       ------------------------------------------------------------
BASF-Gm         AAGCAGTGGTAACAACGCAGAGTACGCGGGGGGAGACGCATGGTTCTGAACTAATTGTTA
AT4g01320       ------------------------------------------------------------
AF007269        CTGCAACTAGAGTTTTCTGGAGTTTTTTGAAATGGGTTTTGTGTTGTGGAACCGTATGTG
PPI-AtCPP       ------------------------------------------------------------
BASF_AT2        ------------------------------------------------------------
afc1            ------------------------------------------------------------
BASF_AT1        ------------------------------------------------------------
PPI-BnCPP       ------------------------------------------------------------
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BASF-Corn       ------------------------------------------------------------

PPI-GmCPP       ------------------------------------------------------------
BASF-Gm         TAAATAATACCTAAAATTTTGAGTTGTCCTAAACATTGGGGTTTAAACAAATCCAATCTC
AT4g01320       ------------------------------------------------------------
AF007269        AATGTTGCATCAAAACTCTTTCAGTGCTCCAATGTTTCCATCAGTAGTCAGCACAAGAGA
PPI-AtCPP       ------------------------------------------------------------
BASF_AT2        ------------------------------------------------------------
afc1            ------------------------------------------------------------
BASF_AT1        ------------------------------------------------------------
PPI-BnCPP       ------------------------------------------------------------
BASF-Corn       ------------------------------------------------------------

PPI-GmCPP       ------------------------------------------------------------
BASF-Gm         TCAATATAAAACCCAATGATCTCACC--CTCACTCCGTTTCTGATTTCTCACTCTTCGTT
AT4g01320       ------------------------------------------------------------
AF007269        TCTTTTTATATCTGGTTGATCAAAAAAGTAGATGATGTTATTGAATTTTCAGTGATGGAG
PPI-AtCPP       ------------------------------------------------------------
BASF_AT2        ------------------------------------------------------------
afc1            ------------------------------------------------------------
BASF_AT1        ------------------------------------------------------------
PPI-BnCPP       ------------------------------------------------------------
BASF-Corn       ------------------------------------------------------------

PPI-GmCPP       ---------------------------------ATGGCGTTTCCC--TACATGGAAGCCG
BASF-Gm         TCTCGTTCGGTTCATCAGCGTGTGTCTCAGC-CATGGCGTTTCCC--TACATGGAAGCCG
AT4g01320       ---------------------------------ATGGCGATTCCT--TTCATGGAAACCG
AF007269        TATCTGTTGTTGTGGCATTTAGAGTAGATTCGTATTTCATCTTCTGTTTTATTCTTTTTC
PPI-AtCPP       ---------------------------------ATGGCGATTCCT--TTCATGGAAACCG
BASF_AT2        ---------------------------------ATGGCGATTCCT--TTCATGGAAACCG
afc1            ---------------------------------ATGGCGATTCCT--TTCATGGAAACCG
BASF_AT1        ---------------------------------ATGGCGATTCCT--TTCATGGAAACCG
PPI-BnCPP       ---------------------------------ATGGCGATTCCT--TTCATGGAAACCG
BASF-Corn       ------------------------------------------------------------

PPI-GmCPP       TTGTCGGATTTATGATATTAATGTACATTTTTGAAACTTACTTGGATGTGCGACAACATA
BASF-Gm         TTGTCGGATTTATGATATTAATGTACATTTTTGAAACTTACTTGGATGTGCGACAACATA
AT4g01320       TCGTGGGTTTTATGATAGTGATGTACATTTTTGAGACGTATTTGGATCTGAGGCAACTCA
AF007269        TTACAGGTTTTATGATAGTGATGTACATTTTTGAGACGTATTTGGATCTGAGGCAACTCA
PPI-AtCPP       TCGTGGGTTTTATGATAGTGATGTACATTTTTGAGACGTATTTGGATCTGAGGCAACTCA
BASF_AT2        TCGTGGGTTTTATGATAGTGATGTACATTTTTGAGACGTATTTGGATCTGAGGCAACTCA
afc1            TCGTGGGTTTTATGATAGTGATGTACATTTTTGAGACGTATTTGGATCTGAGGCAACTCA
BASF_AT1        TCGTGGGTTTTATGATAGTGATGTACATTTTTGAGACGTATTTGGATCTGAGGCAACTCA
PPI-BnCPP       TCGTTGGTTTTATGATAGTGATGTACGTTTTTGAGACGTATTTGGATCTGAGGCAACATA
BASF-Corn       ------------------------------------------------------------

PPI-GmCPP       GGGCCCTCAAACTTCCTACTCTTCCAAAGACTTTAGAGGGTGTTATCAGCCAAGAGAAAT
BASF-Gm         GGGCCCTCAAACTTCCTACTCTTCCAAAGACTTTAGAAGGTGTTATCAGCCAAGAGAAAT
AT4g01320       CTGCTCTCAAGCTTCCAACTCTCCCGAAAACCTTGGTTGGTGTAATTAGCCAAGAGAAGT
AF007269        CTGCTCTCAAGCTTCCAACTCTCCCGAAAACCTTGGTTGGTGTAATTAGCCAAGAGAAGT
PPI-AtCPP       CTGCTCTCAAGCTTCCAACTCTCCCGAAAACCTTGGTTGGTGTAATTAGCCAAGAGAAGT
BASF_AT2        CTGCTCTCAAGCTTCCAACTCTCCCGAAAACCTTGGTTGGTGTAATTAGCCAAGAGAAGT
afc1            CTGCTCTCAAGCTTCCAACTCTCCCGAAAACCTTGGTTGGTGTAATTAGCCAAGAGAAGT
BASF_AT1        CTGCTCTCAAGCTTCCAACTCTCCCGAAAACCTTGGTTGGTGTAATTAGCCAAGAGAAGT
PPI-BnCPP       CTGCTCTCAAGCTTCCCACTCTCCCAAAGACTTTGGTTGGAGTCATTAGCCAAGAGAAGT
BASF-Corn       ------------------------------------------------------------

PPI-GmCPP       TTGAGAAATCTAGAGCCTATAG----------------------------------------
BASF-Gm         TTGAGAAATCTAGAGCCTATAG----------------------------------------
AT4g01320       TTGAGAAATCACGAGCATACAG----------------------------------------
AF007269        TTGAGAAATCACGAGCATACAGTCTTGACAAAAGGTTTCGTCTTGATCATATTTATATCA
PPI-AtCPP       TTGAGAAATCACGAGCATACAG----------------------------------------
BASF_AT2        TTGAGAAATCACGAGCATACAG----------------------------------------
afc1            TTGAGAAATCACGAGCATACAG----------------------------------------
BASF_AT1        TTGAGAAATCACGAGCATACAG----------------------------------------
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PPI-BnCPP       TTGAGAAATCTCGAGCTTACAG-----------------------------------
BASF-Corn       ------------------------------------------------------------


PPI-GmCPP       ----------------------------------------TCTTGATAAA---AGCCA
BASF-Gm         ----------------------------------------TCTTGATAAA---AGCCA
AT4g01320       ----------------------GGATATCATCACTGAGAACTTTAATATATGCAGCTA
AF007269        TTTTAGTTTTTTATAATTGCCAGGGGATATCATCACTGAGAACTTTAATATATGCAGCTA
PPI-AtCPP       ----------------------------------------TCTTGACAAA---AGCTA
BASF_AT2        ----------------------------------------TCTTGACAAA---AGCTA
afc1            ----------------------------------------TCTTGACAAA---AGCTA
BASF_AT1        ----------------------------------------TCTTGACAAA---AGCTA
PPI-BnCPP       ----------------------------------------TCTTGACAAA---AGCCA
BASF-Corn       ------------------------------------------------------------

PPI-GmCPP       CTTCCATTTTGTTCACGAGTTTGTGACAATAGTGACAGACTCTACAATTTTGTACTTTGG
BASF-Gm         CTTCCATTTTGTTCACGAGTTTGTGACAATAGTGACAGACTCTACAATTTTGTACTTTGG
AT4g01320       TTTTCACTTTGTTCATGAGTTTGTAACTATACTTATGGACTCTGCAATTTTGTTCTTTGG
AF007269        TTTTCACTTTGTTCATGAGTTTGTAACTATACTTATGGACTCTGCAATTTTGTTCTTTGG
PPI-AtCPP       TTTTCACTTTGTTCATGAGTTTGTAACTATACTTATGGACTCTGCAATTTTGTTCTTTGG
BASF_AT2        TTTTCACTTTGTTCATGAGTTTGTAACTATACTTATGGACTCTGCAATTTTGTTCTTTGG
afc1            TTTTCACTTTGTTCATGAGTTTGTAACTATACTTATGGACTCTGCAATTTTGTTCTTTGG
BASF_AT1        TTTTCACTTTGTTCATGAGTTTGTAACTATACTTATGGACTCTGCAATTTTGTTCTTTGG
PPI-BnCPP       TTTTCACTTTGTTCATGAGTTTGTTACTATACTTATGGACTCTGCGATTCTGTTCTTTGG
BASF-Corn       ------------------------------------------------------------

PPI-GmCPP       GGTATTGCCCTGGTTTTGGAAG-------------------------------------
BASF-Gm         GGTATTGCCCTGGTTTTGGAAG-------------------------------------
AT4g01320       GATCTTGCCTTGGTTTTGGAAG-------------------------------------
AF007269        GATCTTGCCTTGGTTTTGGAAGGTACATATCTGGTTTCGGTATACAGTATCTCATTTTGA
PPI-AtCPP       GATCTTGCCTTGGTTTTGGAAG-------------------------------------
BASF_AT2        GATCTTGCCTTGGTTTTGGAAG-------------------------------------
afc1            GATCTTGCCTTGGTTTTGGAAG-------------------------------------
BASF_AT1        GATCTTGCCTTGGTTTTGGAAG-------------------------------------
PPI-BnCPP       GATCTTGCCTTGGTTTTGGAAG-------------------------------------
BASF-Corn       ------------------------------------------------------------

PPI-GmCPP       ------------------------------------------------AAATCAGGAGAT
BASF-Gm         ------------------------------------------------AAATCAGGAGAT
AT4g01320       ------------------------------------------------ATGTCTGGAGCT
AF007269        ATATAGAGTTGTTACATTACAATTGTAAAGTTTTCATTTTTACCTTAGATGTCTGGAGCT
PPI-AtCPP       ----------------------------------------------ATGTCTGGAGCT
BASF_AT2        ----------------------------------------------ATGTCTGGAGCA
afc1            ----------------------------------------------ATGTCTGGAGCT
BASF_AT1        ----------------------------------------------ATGTCTGGAGCT
PPI-BnCPP       ----------------------------------------------ATATCTGGCGGC
BASF-Corn       ------------------------------------------------------------
PPI-GmCPP       TTTATGACAATAGCTGGTTTCAATGCTGAGAATGAAATACTGCATACCCTTGCCTTCTTA
BASF-Gm         TTTATGACAATAGCTGGTTTCAATGCTGAGAATGAAATACTGCATACCCTTGCCTTCTTA
AT4g01320       GTTTTACCGAGGTTGGGCCTTGATCCAGAGAATGAAATACTGCATACTCTTTCATTCTTG
AF007269        GTTTTACCGAGGTTGGGCCTTGATCCAGAGAATGAAATACTGCATACTCTTTCATTCTTG
PPI-AtCPP       GTTTTACCGAGGTTGGGCCTTGATCCGGAGAATGAAATACTGCATACTCTTTCATTCTTG
BASF_AT2        GTTTTACCGAGGTTGGGCCTTGATCCAGAGAATGAAATACTGCATACTCTTTCATTCTTG
afc1            GTTTTACCGAGGTTGGGCCTTGATCCAGAGAATGAAATACTGCATACTCTTTCATTCTTG
BASF_AT1        GTTTTACCGAGGTTGGGCCTTGATCCAGAGAATGAAATACTGCATACTCTTTCATTCTTG
PPI-BnCPP       TTTCTACCAATGGTGGGACTCGATCCAGAGAATGAAATCCTGCACACTCTTTCATTCTTG
BASF-Corn       ------------ACGAGGCTGAGTGCTGAGAATGAGATAATACACACCCTTGCTTTCTTA
                             *  *   * * ******** **  * ** ** *** * *****
PPI-GmCPP       GCAGGGCTGATGATTTGGTCACAG-----------------------------------
BASF-Gm         GCAGGGCTGATGATTTGGTCACAG-----------------------------------
AT4g01320       GCTGGTGTTATGACATGGTCACAG-----------------------------------
AF007269        GCTGGTGTTATGACATGGTCACAGGTGTTCCAAATAAACCCCTTCATATAGTCCTATACG
PPI-AtCPP       GCTGGTGTTATGACATGGTCACAG-----------------------------------
BASF_AT2        GCTGGTGTTATGACATGGTCACAG-----------------------------------
afc1            GCTGGTGTTATGACATGGTCACAG-----------------------------------
BASF_AT1        GCTGGTGTTATGACATGGTCACAC-----------------------------------
PPI-BnCPP       GCTGGTCTTATGACATGGTCACAG-----------------------------------
BASF-Corn       GCTGGTTCCATGGTTTGGTCGCAG-----------------------------------
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                ** **    ***   ***** **

PPI-GmCPP       ------------------------------------------------------------
BASF-Gm         ------------------------------------------------------------
AT4g01320       ------------------------------------------------------------
AF007269        TTTAGCATCAAAATATCTATTTTCTTAAGATAATAATATTTCTTTTATATTCTGATGCAG
PPI-AtCPP       ------------------------------------------------------------
BASF_AT2        ------------------------------------------------------------
afc1            ------------------------------------------------------------
BASF_AT1        ------------------------------------------------------------
PPI-BnCPP       ------------------------------------------------------------
BASF-Corn       ------------------------------------------------------------

PPI-GmCPP       ATAACAGATTTGCCCTTTTCTCTGTACTCAACTTTTGTGATTGAGGCCCGTCATGGTTTT
BASF-Gm         ATAACAGATTTGCCCTTTTCTCTGTACTCAACTTTTGTGATTGAGGCCCGTCATGGTTTT
AT4g01320       ATCACTGATTTGCCATTTTCTTTGTACTCAACTTTCGTGATCGAGTCTCGGCATGGGTTC
AF007269        ATCACTGATTTGCCATTTTCTTTGTACTCAACTTTCGTGATCGAGTCTCGGCATGGGTTC
PPI-AtCPP       ATCACTGATTTGCCATTTTCTTTGTACTCAACTTTCGTGATCGAGTCTCGGCATGGGTTC
BASF_AT2        ATCACTGATTTGCCATTTTCTTTGTACTCAACTTTCGTGATCGAGTCTCGGCATGGGTTC
afc1            ATCACTGATTTGCCATTTTCTTTGTACTCAACTTTCGTGATCGAGTCTCGGCATGGGTTC
BASF_AT1        ATCACTGATTTGCCATTTTCTTTGTACTCAACTTTCGTGATCGAGTCTCGGCATGGGTTC
PPI-BnCPP       ATCACTGATTTGCCATTTTCTTTGTACTCAACTTTCGTGATCGAGTCTCGGCATGGGTTC
BASF-Corn       ATTACAGACTTGCCGTTCTCTCTCTATTCAACTTTTGTTATAGAGGCTCGACATGGTTTT
                ** ** ** ***** ** *** * ** ******** ** ** *** * ** ***** **

PPI-GmCPP       AATAAG------------------------------------------------------
BASF-Gm         AATAAG------------------------------------------------------
AT4g01320       AACAAA------------------------------------------------------
AF007269        AACAAAGTATGTCGTATTTCCAACACTACCTTGTGACTTACGTTTTTTTATCAGAGATGT
PPI-AtCPP       AACAAA------------------------------------------------------
BASF_AT2        AACAAA------------------------------------------------------
afc1            AACAAA------------------------------------------------------
BASF_AT1        AACAAA------------------------------------------------------
PPI-BnCPP       AACAAA------------------------------------------------------
BASF-Corn       AACAAG------------------------------------------------------
                ** **
PPI-GmCPP       --------------------------------CAAACACCATGGTTATTCTTTAGGGACA
BASF-Gm         --------------------------------CAAACACCATGGTTATTCTTTAGGGACA
AT4g01320       --------------------------------CAAACAATATGGATGTTCATTAGGGACA
AF007269        GGATTAAATTTGCTTCTAAATTCTGTTGACAGCAAACAATATGGATGTTCATTAGGGACA
PPI-AtCPP       --------------------------------CAAACAATATGGATGTTCATTAGGGACA
BASF_AT2        --------------------------------CAAACAATATGGATGTTCATTAGGGACA
afc1            --------------------------------CAAACAATATGGATGTTCATTAGGGACA
BASF_AT1        --------------------------------CAAACAATATGGATGTTCATTAGGGACA
PPI-BnCPP       --------------------------------CAAACAATATGGATGTTCATTAGGGACA
BASF-Corn       --------------------------------CAAACTATATGGCTCTTCATTAGGGATA
                                                *****   **** * *** ******* *

PPI-GmCPP       TGCTTAAAGGAATTTTCCTTTCTGTAATAATTGGTCCACCTATTGTGGCTGCAATCATTG
BASF-Gm         TGCTTAAAGGAATTTTCCTTTCCGTAATAATTGGTCCACCTATTGTGGCTGCAATCATTG
AT4g01320       TGATCAAAGGAACATTCCTCTCTGTCATACTAGGCCCACCCATTGTTGCTGCGATAATTT
AF007269        TGATCAAAGGAACATTCCTCTCTGTCATACTAGGCCCACCCATTGTTGCTGCGATAATTT
PPI-AtCPP       TGATCAAAGGAACATTCCTCTCTGTCATACTAGGCCCACCCATTGTTGCTGCGATAATTT
BASF_AT2        TGATCAAAGGAACATTCCTCTCTGTCATACTAGGCCCACCCATTGTTGCTGCGATAATTT
afc1            TGATCAAAGGAACATTCCTCTCTGTCATACTAGGCCCACCCATTGTTGCTGCGATAATTT
BASF_AT1        TGATCAAAGGAACATTCCTCTCTGTCATACTAGGCCCACCCATTGTTGCCGCGATAATTT
PPI-BnCPP       TGATCAAAGGAATACTCCTCTCTGTCATACCTGCCCCTCCTATCGTTGCCGCAATTATTG
BASF-Corn       TGATCAAAGGAATTTTACTATCCATGATATTGGGGCCACCAATCGTGGCTGCTATCATCT
                ** * *******   * ** **  * ***   *  ** ** ** ** ** ** ** **

PPI-GmCPP       TAATAGTACAG-------------------------------------------------
BASF-Gm         TAATAGTACAG-------------------------------------------------
AT4g01320       TCATAGTCCAG-------------------------------------------------
AF007269        TCATAGTCCAGGTTTGATGATTCTGGATTCATCTTATTTCTGAGTTTTTCACATGGATGA
PPI-AtCPP       TCATAGTCCAG-------------------------------------------------
BASF_AT2        TCATAGTCCAG-------------------------------------------------
afc1            TCATAGTCCAG-------------------------------------------------
BASF_AT1        TCATAGTCCAG-------------------------------------------------
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PPI-BnCPP       TTATAGTTCAG-----------------------------------------------
BASF-Corn       ACATAGTACAG-----------------------------------------------
                  ***** ***
PPI-GmCPP       ------------------------------------------------------------
BASF-Gm         ------------------------------------------------------------
AT4g01320       ------------------------------------------------------------
AF007269        CTATTCTCCATTGAGTGTGAGCTTCAAAGTTTTTAGTTTTCGTGTTAAAAATTTAAAATT
PPI-AtCPP       ------------------------------------------------------------
BASF_AT2        ------------------------------------------------------------
afc1            ------------------------------------------------------------
BASF_AT1        ------------------------------------------------------------
PPI-BnCPP       ------------------------------------------------------------
BASF-Corn       ------------------------------------------------------------

PPI-GmCPP       ----------------------------------AAAGGAGGTCCATACTTGGCCATC
BASF-Gm         ----------------------------------AAAGGAGGTCCATACTTGGCCATC
AT4g01320       ----------------------------------AAAGGAGGTCCTTATCTTGCCATC
AF007269        TGCTTCTCTGAGCATGAAGTTTCTATCTTTTTCCAGAAAGGAGGTCCTTATCTTGCCATC
PPI-AtCPP       ----------------------------------AAAGGAGGTCCTTATCTTGCCATC
BASF_AT2        ----------------------------------AAAGGAGGTCCTTATCTTGCCATC
afc1            ----------------------------------AAAGGAGGTCCTTATCTTGCCATC
BASF_AT1        ----------------------------------AAAGGAGGTCCTTATCTTGCCATC
PPI-BnCPP       ----------------------------------AAAGGAGGTCCTTACCTCGCCATC
BASF-Corn       ----------------------------------ATTGGAGGACCTTACCTGGCTATA
                                                   *  ***** ** **  * ** **
PPI-GmCPP       TATCTTTGGGTTTTTACGTTTGGTCTTTCTATTGTGATGATGACCCTTTATCCAGTACTA
BASF-Gm         TATCTTTGGGTTTTTACGTTTGGTCTTTCTATTGTGATGATGACCCTTTATCCAGTACTA
AT4g01320       TATCTGTGGGCATTCATGTTTATCCTGTCTCTAGTGATGATGACTATATACCCGGTCTTG
AF007269        TATCTGTGGGCATTCATGTTTATCCTGTCTCTAGTGATGATGACTATATACCCGGTCTTG
PPI-AtCPP       TATCTGTGGGCATTCATGTTTATCCTGTCTCTAGTGATGATGACTATATACCCGGTCTTG
BASF_AT2        TATCTGTGGGCATTCATGTTTATCCTGTCTCTAGTGATGATGACTATATACCCGGTCTTG
afc1            TATCTGTGGGCATTCATGTTTATCCTGTCTCTAGTGATGATGACTATATACCCGGTCTTG
BASF_AT1        TATCTGTGGGCATTCATGTTTATCCTGTCTCTAGTGATGATGACTATATACCCGGTCTTG
PPI-BnCPP       TATCTGTGGGCATTCATGTTTATCCTGTCTCTAGTGATGATGACTATATACCCTGTTTTG
BASF-Corn       TATCTCTGGGGTTTTATGTTTGTATTAGCTCTACTGATGATGACAATATACCCCATTGTG
                ***** ****  ** * ****    *  ** *  ********** * ** **  *  *

PPI-GmCPP       ATAGCTCCACTCTTCAATAAGTTCACTCCA----------------------------
BASF-Gm         ATAGCTCCACTCTTCAATAAGTTCACTCCA----------------------------
AT4g01320       ATAGCACCGCTCTTCAACAAGTTCACTCCT----------------------------
AF007269        ATAGCACCGCTCTTCAACAAGTTCACTCCTGTGTGTATTTCTGTCATGGCCATTTTACAA
PPI-AtCPP       ATAGCACCGCTCTTCAACAAATTCACTCCT----------------------------
BASF_AT2        ATAGCACCGCTCTTCAACAAGTTCACTCCT----------------------------
afc1            ATAGCACCGCTCTTCAACAAGTTCACTCCT----------------------------
BASF_AT1        ATAGCACCGCTCTTCAACAAGTTCACTCCT----------------------------
PPI-BnCPP       ATTGCACCTCTTTTCAACAAGTTCACTCCT----------------------------
BASF-Corn       ATAGCTCCTCTGTTCAACAAGTTCACTCCT----------------------------
                ** ** ** ** ***** ** ********
PPI-GmCPP       ------------------------------------------------------------
BASF-Gm         ------------------------------------------------------------
AT4g01320       ------------------------------------------------------------
AF007269        TTCACTGCTTGTTTGCATATGTTGTTACCAGACAATATAATCTCCCGCTTTTTTATGGCT
PPI-AtCPP       ------------------------------------------------------------
BASF_AT2        ------------------------------------------------------------
afc1            ------------------------------------------------------------
BASF_AT1        ------------------------------------------------------------
PPI-BnCPP       ------------------------------------------------------------
BASF-Corn       ------------------------------------------------------------

PPI-GmCPP       ----CTTCCAGATGGTCAACTCAGGGAGAAAATCGAGAAACTTGCTTCCTCCCTCAACTA
BASF-Gm         ----CTTCCAGATGGTCAACTCAGGGAGAAAATCGAGAAACTTGCTTCCTCCCTCAACTA
AT4g01320       ----CTTCCAGATGGAGACCTCCGGGAGAAGATTGAGAAACTTGCTTCTTCTCTAAAGTT
AF007269        ATAGCTTCCAGATGGAGACCTCCGGGAGAAGATTGAGAAACTTGCTTCTTCTCTAAAGTT
PPI-AtCPP       ----CTTCCAGATGGAGACCTCCGGGAGAAGATTGAGAAACTTGCTTCTTCCCTAAAGTT
BASF_AT2        ----CTTCCAGATGGAGACCTCCGGGAGAAGATTGAGAAACTTGCTTCTTCTCTAAAGTT
afc1            ----CTTCCAGATGGAGACCTCCGGGAGAAGATTGAGAAACTTGCTTCTTCTCTAAAGTT
BASF_AT1        ----CTTCCAGATGGAGACCTCCGGGAGAAGATTGAGAAACTTGCTTCTTCTCTAAAGTT
PPI-BnCPP       ----CTTCCTGATGGAGACCTCCGGGAGAAGATTGAGAAACTTGCTTCTTCTCTAAAGTT
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BASF-Corn       ----CTTCCTGAAGGAGTCCTCAGGGAAAAAATAGAGAAGCTGGCAGCTTCCCTCAAGTT
                    ***** ** **    *** **** ** ** ***** ** **  * ** ** ** *

PPI-GmCPP       TCCGTTAAAGAAACTATTTGTTGTCGATGGATCCACAAGATCAAGTCACAGCAATG----
BASF-Gm         TCCGTTAAAGAAACTATTTGTTGTCGATGGATCCACAAGATCAAGTCACAGCAATG----
AT4g01320       TCCTTTGAAGAAGCTGTTTGTTGTCGATGGATCTACAAGGTCAAGCCATAGCAATG----
AF007269        TCCTTTGAAGAAGCTGTTTGTTGTCGATGGATCTACAAGGTCAAGCCATAGCAATGTGAG
PPI-AtCPP       TCCTTTGAAGAAGCTGTTTGTTGTCGATGGATCTACAAGGTCAAGCCATAGCAATG----
BASF_AT2        TCCTTTGAAGAAGCTGTTTGTTGTCGATGGATCTACAAGGTCAAGCCATAGCAATG----
afc1            TCCTTTGAAGAAGCTGTTTGTTGTCGATGGATCTACAAGGTCAAGCCATAGCAATG----
BASF_AT1        TCCTTTGAAGAAGCTGTTTGTTGTCGATGGATCTACAAGGTCAAGCCATAGCAATG----
PPI-BnCPP       TCCTCTGAAGAAGCTGTTTGTTGTCGATGGATCTACAAGGTCAAGCCATAGTAATG----
BASF-Corn       TCCTTTGAAAAAGCTTTTCGTGGTAGATGGGTCTACCAGATCAAGCCACAGTAATG----
                ***  * ** ** ** ** ** ** ** ***** ** ** ** ***** ** ** ****

PPI-GmCPP       ------------------------------------------------------------
BASF-Gm         ------------------------------------------------------------
AT4g01320       ------------------------------------------------------------
AF007269        AAGCTTGAGATCTCTTCCTACCTACTTTACTCTAGTTTACCATTAGAAGCTTACGTATCT
PPI-AtCPP       ------------------------------------------------------------
BASF_AT2        ------------------------------------------------------------
afc1            ------------------------------------------------------------
BASF_AT1        ------------------------------------------------------------
PPI-BnCPP       ------------------------------------------------------------
BASF-Corn       ------------------------------------------------------------

PPI-GmCPP       ----------------CCTATATGTATGGATTCTTCAAGAACAAGAGGATTGTCCCTTAT
BASF-Gm         ----------------CCTATATGTATGGATTCTTCAAGAACAAGAGGATTGTCCTTTAT
AT4g01320       ----------------CTTACATGTATGGTTTCTTTAAGAACAAAAGGATTGTTCTTTAT
AF007269        TGTTACATCATACAGGCTTACATGTATGGTTTCTTTAAGAACAAAAGGATTGTTCTTTAT
PPI-AtCPP       ----------------CTTACATGTATGGTTTCTTTAAGAACAAAAGGATTGTTCTTTAT
BASF_AT2        ----------------CTTACATGTATGGTTTCTTTAAGAACAAAAGGATTGTTCTTTAT
afc1            ----------------CTTACATGTATGGTTTCTTTAAGAACAAAAGGATTGTTCTTTAT
BASF_AT1        ----------------CTTACATGTATGGTTTCTTTAAGAACAAAAGGATTGTTCTTTAT
PPI-BnCPP       ----------------CTTACATGTATGGTTTCTTCAAGAACAAAAGGATTGTTCTTTAT
BASF-Corn       ----------------CCTACATGTATGGTTTTTTCAAGAACAAGCGCATAGTACTCTAT
                               * ** ******** ** ** ********  * ** ** *  ***

PPI-GmCPP       GACACATTAATTCAACAG------------------------------------------
BASF-Gm         GACACATTAATTCAACAG------------------------------------------
AT4g01320       GATACGTTGATTCAGCAG------------------------------------------
AF007269        GATACGTTGATTCAGCAGGTACTGTGACTCTTGATGCTTCAAACGAGCTATACTCACATT
PPI-AtCPP       GATACGTTGATTCAGCAG------------------------------------------
BASF_AT2        GATACGTTGATTCAGCAG------------------------------------------
afc1            GATACGTTGATTCAGCAG------------------------------------------
BASF_AT1        GATACGTTGATTCAGCAG------------------------------------------
PPI-BnCPP       GACACATTGATTCAGCAG------------------------------------------
BASF-Corn       GACACATTGATTCAGCAG------------------------------------------
                ** ** ** ***** ***

PPI-GmCPP       ------------------------------------------TGCAAAGACGATGAGG
BASF-Gm         ------------------------------------------TGCAAAGACGATGAGG
AT4g01320       ------------------------------------------TGCAAGAATGAGGATG
AF007269        TCTGTTTCTGGTTCTGAAACATAACATAATCTTCTATTGTGCAGTGCAAGAATGAGGATG
PPI-AtCPP       ------------------------------------------TGCAAGAATGAGGATG
BASF_AT2        ------------------------------------------TGCAAGAATGAGGATG
afc1            ------------------------------------------TGCAAGAATGAGGATG
BASF_AT1        ------------------------------------------TGCAAGAATGAGGATG
PPI-BnCPP       ------------------------------------------TGCCAGAATGAGAATG
BASF-Corn       ------------------------------------------TGTAGCAATGAGGATG
                                                           **     * **  * *

PPI-GmCPP       AAATTGTTGCTGTTATTGCCCATGAGTTGGGACACTGGAAGCTCAACCATACTGTGTACA
BASF-Gm         AAATTGTTGCTGTTATTGCCCATGAGTTGGGACACTGGAAGCTCAACCATACTGTGTACA
AT4g01320       AAATTGTGGCGGTTATTGCACACGAGCTTGGACATTGGAAACTGAATCACACTACATACT
AF007269        AAATTGTGGCGGTTATTGCACACGAGCTTGGACATTGGAAACTGAATCACACTACATACT
PPI-AtCPP       AAATTGTGGCGGTTATTGCACACGAGCTTGGACATTGGAAACTGAATCACACTACATACT
BASF_AT2        AAATTGTGGCGGTTATTGCACACGAGCTTGGACATTGGAAACTGAATCACACTACATACT
afc1            AAATTGTGGCGGTTATTGCACACGAGCTTGGACATTGGAAACTGAATCACACTACATACT
BASF_AT1        AAATTGTGGCGGTTATTGCACACGAGCTTGGACATTGGAAACTGAATCACACTACATACT
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PPI-BnCPP       AAATTGTGGCGGTTATTGCACACGAGCTGGGACACTGGAAGCTGAATCACACTACATACT
BASF-Corn       AGATAGTTTCTGTTATAGCACATGAACTTGGACACTGGAAACTCAATCATACTGTCTATT
                * ** **  * ***** ** ** **  * ***** ***** ** ** ** ***   **
PPI-GmCPP       CATTTGTTGCTATGCAG----------------------------------------
BASF-Gm         CATTTGTTGCTATGCAG----------------------------------------
AT4g01320       CGTTCATTGCAGTTCAA----------------------------------------
AF007269        CGTTCATTGCAGTTCAAGTGAGGCTCAACCGACAGTTCAAAAACTTACTCACATCTACAT
PPI-AtCPP       CGTTCATTGCAGTTCAA----------------------------------------
BASF_AT2        CGTTCATTGCAGTTCAA----------------------------------------
afc1            CGTTCATTGCAGTTCAA----------------------------------------
BASF_AT1        CGTTCATTGCAGTTCAA----------------------------------------
PPI-BnCPP       CGTTCATTGCTGTTCAA----------------------------------------
BASF-Corn       CCTTTGTAGCTGTCCAG----------------------------------------
                * **  * **  * **
PPI-GmCPP       ----------------------------------------------------ATTCTTACA
BASF-Gm         ----------------------------------------------------ATTCTTACA
AT4g01320       ----------------------------------------------------ATCCTTGCC
AF007269        TTCACTTAAGAAATCATGTCTTATGACCCTCTCTCAATGTTTTGCTTGCAGATCCTTGCC
PPI-AtCPP       ----------------------------------------------------ATCCTTGCC
BASF_AT2        ----------------------------------------------------ATCCTTGCC
afc1            ----------------------------------------------------ATCCTTGCC
BASF_AT1        ----------------------------------------------------ATCCTTGCC
PPI-BnCPP       ----------------------------------------------------ATCCTTGCC
BASF-Corn       ----------------------------------------------------CTGCTTATG
                                                                     * ***

PPI-GmCPP       CTTCTACAATTTGGAGGATATACACTAGTGCGAAATTCAGCTGATCTGTATCGAAGCTTT
BASF-Gm         CTTCTACAATTTGGAGGATATACACTAGTGCGAAATTCAGCTGATCTGTATCGAAGCTTT
AT4g01320       TTCTTACAATTTGGAGGATACACTCTTGTCAGAAACTCCACTGATCTCTTCAGGAGTTTC
AF007269        TTCTTACAATTTGGAGGATACACTCTTGTCAGAAACTCCACTGATCTCTTCAGGAGTTTC
PPI-AtCPP       TTCTTACAATTTGGAGGATACACTCTTCTCAGAAACTCCACTGATCTCTTCAGGAGTTTC
BASF_AT2        TTCTTACAATTTGGAGGATACACTCTTGTCAGAAACTCCACTGATCTCTTCAGGAGTTTC
afc1            TTCTTACAATTTGGAGGATACACTCTTGTCAGAAACTCCACTGATCTCTTCAGGAGTTTC
BASF_AT1        TTCTTACAATTTGGAGGATACACTCTTGTCAGAAACTCCACTGATCTCTTCAGGAGTTTC
PPI-BnCPP       TTCTTGCAATTTGGAGGATACACTCTTGTCAGAAACTCCACTGATCTCTTCAGGAGTTTT
BASF-Corn       TTTCTTCAATTTGGAGGATATACTCTAGTAAGGAGCTCCAAAGATCTATTTGGAAGTTTT
                 *  * ************** ** **  *  * *  **    ***** *   * ** **

PPI-GmCPP       GGGTTTGATACGCAGCCAGTCCTCATTGGGCTCATCATATTTCAG---------------
BASF-Gm         GGGTTTGATACGCAGCCAGTCCTCATTGGGCTCATCATATTTCAG---------------
AT4g01320       GGATTTGATACACAGCCTGTTCTCATTGGTTTGATCATATTTCAG---------------
AF007269        GGATTTGATACACAGCCTGTTCTCATTGGTTTGATCATATTTCAGGTTTGTTATTTTTGC
PPI-AtCPP       GGATTTGATACACAGCCTGTTCTCATTGGTTTGATCATATTTCAG---------------
BASF_AT2        GGATTTGATACACAGCCTGTTCTCATTGGTTTGATCATATTTCAG---------------
afc1            GGATTTGATACACAGCCTGTTCTCATTGGTTTGATCATATTTCAG---------------
BASF_AT1        GGATTTGATACACAGCCTGTTCTCATTGGTTTGATCATATTTCAG---------------
PPI-BnCPP       GGTTTTGATACACAACCAGTTCTCATTGGTTTGATCATATTTCAG---------------
BASF-Corn       GGCTTCAAGGACCAGCCAGTAATAATTGGATTGATCATTTTCCCG---------------
                ** **  *    ** ** **  * *****  * ***** ** * *

PPI-GmCPP       ----------------------------------------------------------
BASF-Gm         ----------------------------------------------------------
AT4g01320       ----------------------------------------------------------
AF007269        CTTTTGACACTAATCTAATGAATCAAGGATGGATTAAGAAAAAAAAACTCTAAACCTTTG
PPI-AtCPP       ----------------------------------------------------------
BASF_AT2        ----------------------------------------------------------
afc1            ----------------------------------------------------------
BASF_AT1        ----------------------------------------------------------
PPI-BnCPP       ----------------------------------------------------------
BASF-Corn       ----------------------------------------------------------
PPI-GmCPP       --------------------------CATACTGTAATCCCACTTCAGCAATTGGTCAGC
BASF-Gm         --------------------------CATACTGTAATCCCACTTCAGCAATTGGTCAGC
AT4g01320       --------------------------CACACTGTAATACCACTGCAACATCTAGTAAGC
AF007269        GTTATATCTCCTGTCTGATTATCACAGCACACTGTAATACCACTGCAACATCTAGTAAGC
PPI-AtCPP       --------------------------CACACTGTAATACCACTGCAACATCTAGTAAGC
BASF_AT2        --------------------------CACACTGTAATACCACTGCAACATCTAGTAAGC
afc1            --------------------------CACACTGTAATACCACTGCAACATCTAGTAAGC
BASF_AT1        --------------------------CACACTGTAATACCACTGCAACATCCAGTAAGC
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PPI-BnCPP       --------------------------CACACTGTAATACCACTTCAACACCTAGTAAGC
BASF-Corn       --------------------------CACACCATAATACCCATCCAACACCTTCTGAGC
                                          ** **  **** **  * ** **     * ***

PPI-GmCPP       TTTGGTCTGAACCTAGTCAGCCGATCATTTGAATTTCAGG--------------------
BASF-Gm         TTTGGTCTGAACCTAGTCAGCCGATCATTTGAATTTCAGG--------------------
AT4g01320       TTTGGCCTGAACCTCGTTAGTCGAGCGTTTGAGTTTCAGG--------------------
AF007269        TTTGGCCTGAACCTCGTTAGTCGAGCGTTTGAGTTTCAGGTACCATCTTACAATCCCTCA
PPI-AtCPP       TTTGGCCTGAACCTCGTTAGTCGAGCGTTTGAGTTTCAGG--------------------
BASF_AT2        TTTGGCCTGAACCTCGTTAGTCGAGCGTTTGAGTTTCAGG--------------------
afc1            TTTGGCCTGAACCTCGTTAGTCGAGCGTTTGAGTTTCAGG--------------------
BASF_AT1        TTTGGCCTCAACCTTGTTAGTCGAGCGTTTGAGTTTCAGG--------------------
PPI-BnCPP       TTTGACCTCAACCTTGTTAGTCGAGCGTTTGAGTTTCAGG--------------------
BASF-Corn       TTTCGCCTGAACCTTGTCAGCAGAGCATTTGAATTTCAGG--------------------
                ***   ** ***** ** **  ** * ***** *******
PPI-GmCPP       ----------------------------------------------------------
BASF-Gm         ----------------------------------------------------------
AT4g01320       ----------------------------------------------------------
AF007269        AGATCCAACCATAGTTTCTTTATTGCAATGGCAGCCTCATCTACTAATCTGAGTTAACGT
PPI-AtCPP       ----------------------------------------------------------
BASF_AT2        ----------------------------------------------------------
afc1            ----------------------------------------------------------
BASF_AT1        ----------------------------------------------------------
PPI-BnCPP       ----------------------------------------------------------
BASF-Corn       ----------------------------------------------------------

PPI-GmCPP       ------------CTGATGGCTTTGCCAAGAAGCTTGGATATGCATCTGGATTACGCGGTG
BASF-Gm         ------------CTGATGGCTTTGCCAAGAAGCTTGGATATGCATCTGGATTACGCGGTG
AT4g01320       ------------CTGATGCTTTTGCTGTGAAGCTTGGCTATGCAAAAGATCTTCGTCCTG
AF007269        TCCTTTTGCAGGCTGATGCTTTTGCTGTGAAGCTTGGCTATGCAAAAGATCTTCGTCCTG
PPI-AtCPP       ------------CTGATGCTTTTGCTGTGAAGCTTGACTATGCAAAAGATCTTCGTCCTG
BASF_AT2        ------------CTGATGCTTTTGCTGTGAAGCTTGGCTATGCAAAAGATCTTCGTCCTG
afc1            ------------CTGATGCTTTTGCCGTGAAGCTTGGCTATGCAAAAGATCTTCGTCCTG
BASF_AT1        ------------CTGATGCTTTTGCTGTGAAGCTTGGCTATGCAAAAGATCTTCGTCCTA
PPI-BnCPP       ------------CTGATGCTTTTGCAGTGAATCTTGGTTATGCAAAGGATCTACGTCCTG
BASF-Corn       ------------CTGATGCCTTTGCCAAGAACCTTGGATATGCCCCTCAGCTCCGAGCAG
                            ******  *****   *** ****  *****        * **

PPI-GmCPP       GTCTTGTGAAACTACAGG------------------------------------------
BASF-Gm         GTCTTGTGAAACTACAGG------------------------------------------
AT4g01320       CTCTAGTGAAACTACAGGTCAGAGAAGATAACAACAGAACACAAACTGTTACCTCAATTT
AF007269        CTCTAGTGAAACTACAGGTCAGAGAAGATAACAACAGAACACAAACTGTTACCTCAATTT
PPI-AtCPP       CTCTAGTGAAACTACAGG------------------------------------------
BASF_AT2        CTCTAGTGAAACTACAGG------------------------------------------
afc1            CTCTAGTGAAACTACAGG------------------------------------------
BASF_AT1        CTCTAGTGAAACTACAGG------------------------------------------
PPI-BnCPP       CCCTAGTGAAGCTACAGG------------------------------------------
BASF-Corn       CCCTTGTTAAACTACAGG------------------------------------------
                  ** ** ** *******
PPI-GmCPP       ----------------------------------------AGGAGAATCTGTCAGCTA
BASF-Gm         ----------------------------------------AGGAGAATCTGTCAGCTA
AT4g01320       GTGTCACACACTTAAATGGATTTTTTGTTGGGATTTTGCAGGAAGAGAACTTATCAGCAA
AF007269        GTGTCACACACTTAAATGGATTTTTTGTTGGGATTTTGCAGGAAGAGAACTTATCAGCAA
PPI-AtCPP       ----------------------------------------AAGAGAACTTATCAACAA
BASF_AT2        ----------------------------------------AAGAGAACTTATCAGCAA
afc1            ----------------------------------------AAGAGAACTTATCAGCAA
BASF_AT1        ----------------------------------------AAGAGAACTTATCAGCAA
PPI-BnCPP       ----------------------------------------AAGAGAACTTATCAGCGA
BASF-Corn       ----------------------------------------AGGAGAACTTGTCTGCGA
                                                        * *****  * **  * *

PPI-GmCPP       TGAATACAGATCCTTGGTACTCTGCTTATCACTATTCTCATCCTCCCCTTGTTGAAAGAT
BASF-Gm         TGAATACAGATCCTTGCT--CGTGCCG--------------------------------
AT4g01320       TGAACACTGATCCATTGTACTCAGCTTATCACTACTCACATCCTCCTCTTGTTGAAAGGC
AF007269        TGAACACTGATCCATTGTACTCAGCTTATCACTACTCACATCCTCCTCTTGTTGAAAGGC
PPI-AtCPP       TGAACACTGATCCATTGTACTCAGCTTATCACTACTCACATCCTCCTCTTGTTGAAAGGC
BASF_AT2        TGAAAACTGATCTATTGTACTCAGCTTATCACTACTCACATCCTCCTCTTGTTGAAAGGC
afc1            TGAACACTGATCCATTGCACTCAGCTTATCACTACTCACATCCTCCTCTTGTTGAAAGGC
```

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BASF_AT1        TGAATACTGATCCATTGTACTCAGCTTATCACTACTCACATCCTCCTCTTGTTGAAAGGC
PPI-BnCPP       TGAACACAGACCCATTGTACTCAGCTTATCACTACTCACACCCTCCTCTTGTAGAGAGGC
BASF-Corn       TGAACACCGATCCTTGGTATTCGGCATATCACTACTCCCACCCACCACTCGTCGAGAGGC
                **** ** ** *  *        **

PPI-GmCPP       TGGCCGCGCTGGACGA---ACCGGATAAGAAGGAAGACTAA----------
BASF-Gm         ----------
AT4g01320       TTCGAGCCATTGATGG---AGAAGACAAGAAGACAGATTAA----------
AF007269        TTCGAGCCATTGATGG---AGAAGACAAGAAGACAGATTAA----------
PPI-AtCPP       TTCGAGCCACTGATGG---AGAAGACAAGAAGACAGATTAA----------
BASF_AT2        TTCGAGCCATTGATGG---AGAAGACAAGAAGACAGATTAA----------
afc1            TTCGAGCCATTGATGG---AGAAGACAAGAAGACAGATTAA----------
BASF_AT1        TTCGAGCCATTGATGG---AGAAGACAAGAAGACAGATTAA----------
PPI-BnCPP       TTCGAGCCATTGATGG---AGAAGACAAGAAGACAGATTAA----------
BASF-Corn       TGCAAGCTTTGGAAGATTCAGACGACAAAAAAGAAGATTAGTCGATCCTTGTATGAGGTT

PPI-GmCPP       ----------
BASF-Gm         ----------
AT4g01320       ----------
AF007269        ----------
PPI-AtCPP       ----------
BASF_AT2        ----------
afc1            ----------
BASF_AT1        ----------
PPI-BnCPP       ----------
BASF-Corn       TACATATGGATTTTTCCCTGCCACATGCACACCGATTCAGTGCTTGGATGGTGAGGGTTT

PPI-GmCPP       ----------
BASF-Gm         ----------
AT4g01320       ----------
AF007269        ----------
PPI-AtCPP       ----------
BASF_AT2        ----------
afc1            ----------
BASF_AT1        ----------
PPI-BnCPP       ----------
BASF-Corn       TGACATAGGAGTGTTGTCAAAGCTTTAGAGTGCATCTTTCGGTCAGGTGCAACAGCCTTT

PPI-GmCPP       ----------
BASF-Gm         ----------
AT4g01320       ----------
AF007269        ----------
PPI-AtCPP       ----------
BASF_AT2        ----------
afc1            ----------
BASF_AT1        ----------
PPI-BnCPP       ----------
BASF-Corn       CGGTCATTGAGACATATAAGCGAATTAGCTATTAAAAAAAACAGAACTGTTGCATCAAAA

PPI-GmCPP       ----------
BASF-Gm         ----------
AT4g01320       ----------
AF007269        ----------
PPI-AtCPP       ----------
BASF_AT2        ----------
afc1            ----------
BASF_AT1        ----------
PPI-BnCPP       ----------
BASF-Corn       AAAAAAAAAAAAAAAGAAACAAAAAAAAAAAAAAAAAAAAAAAAGAAAAAAAAAAAAAAAA

PPI-GmCPP       ----------
BASF-Gm         ----------
AT4g01320       ----------
AF007269        ----------
```

```
PPI-AtCPP       --------------------------------------------------
BASF_AT2        --------------------------------------------------
afc1            --------------------------------------------------
BASF_AT1        --------------------------------------------------
PPI-BnCPP       --------------------------------------------------
BASF-Corn       AAAAAGTGCTCTGCGTTGTTACCACTGCTTGCCCTATAGTGATCGTATCAGA
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Table 18B. ClustalW Amino Acid Analysis of CaaX Prenyl Protease

1: PPI-AtCPP SEQ ID NO:98
2: PPI-BnCPP SEQ ID NO:110
3: PPI-GmCPP SEQ ID NO:113
4: BASF_AT1 SEQ ID NO:117
5: BASF_AT2 SEQ ID NO:119
6: BASF-Corn SEQ ID NO:121
7: BASF-Gm SEQ ID NO:123
8: AFC1 SEQ ID NO:125
9: AT4g01320 SEQ ID NO:127
10: AF007269 SEQ ID NO:129

```
PPI-GmCPP       MAFPYMEAVVGFMILMYIFETYLDVRQHRALKLPTLPKTLEG-------VISQEKFEKSR
BASF-Gm         MAFPYMEAVVGFMILMYIFETYLDVRQHRALKLPTLPKTLEG-------VISQEKFEKSR
AF007269        MAIPFMETVVGFMIVMYIFETYLDLRQLTALKLPTLPKTLI--------------------
AT4g-AtCPP      MAIPFMETVVGFMIVMYIFETYLDLRQLTALKLPTLPKTLVGVISQEKFEKSRAYRDIIT
BASF_AT2        MAIPFMETVVGFMIVMYIFETYLDLRQLTALKLPTLPKTLVG-------VISQEKFEKSR
AFC1            MAIPFMETVVGFMIVMYIFETYLDLRQLTALKLPTLPKTLVG-------VISQEKFEKSR
BASF_AT1        MAIPFMETVVGFMIVMYIFETYLDLRQLTALKLPTLPKTLVG-------VISQEKFEKSR
PPI-AtCPP       MAIPFMETVVGFMIVMYIFETYLDLRQLTALKLPTLPKTLVG-------VISQEKFEKSR
PPI-BnCPP       MAIPFMETVVGFMIVMYVFETYLDLRQHTALKLPTLPKTLVG-------VISQEKFEKSR
BASF-Corn       ------------------------------------------------------------


PPI-GmCPP       AYSLDKSHFHFVHEFVTIVTDSTILYFGVLPWFWKKSGDFMTIAGFNAENEILHTLAFLA
BASF-Gm         AYSLDKSHFHFVHEFVTIVTDSTILYFGVLPWFWKKSGDFMTIAGFNAENEILHTLAFLA
AF007269        ------------------------------------------------------------
AT4g-AtCPP      ENFNICSYFHFVHEFVTILMDSAILFFGILPWFWKMSGAVLPRLGLDPENEILHTLSFLA
BASF_AT2        AYSLDKSYFHFVHEFVTILMDSAILFFGILPWFWKMSGAVLPRLGLDPENEILHTLSFLA
AFC1            AYSLDKSYFHFVHEFVTILMDSAILFFGILPWFWKMSGAVLPRLGLDPENEILHTLSFLA
BASF_AT1        AYSLDKSYFHFVHEFVTILMDSAILFFGILPWFWKMSGAVLPRLGLDPENEILHTLSFLA
PPI-AtCPP       AYSLDKSYFHFVHEFVTILMDSAILFFGILPWFWKMSGAVLPRLGLDPENEILHTLSFLA
PPI-BnCPP       AYSLDKSHFHFVHEFVTILMDSAILFFGILPWFWKISGGFLPMVGLDPENEILHTLSFLA
BASF-Corn       ----------------------------------------TRLSAENEIIHTLAFLA


PPI-GmCPP       GLMIWSQITDLPFSLYSTFVIEARHGFNKQTPWLFFRDMLKGIFLSVIIGPPIVAAIIVI
BASF-Gm         GLMIWSQITDLPFSLYSTFVIEARHGFNKQTPWLFFRDMLKGIFLSVIIGPPIVAAIIVI
AF007269        --------TDLPFSLYSTFVIESRHGFNKQTIWMFIRDMIKGTFLSVILGPPIVAAIIFI
AT4g-AtCPP      GVMTWSQITDLPFSLYSTFVIESRHGFNKQTIWMFIRDMIKGTFLSVILGPPIVAAIIFI
BASF_AT2        GVMTWSQITDLPFSLYSTFVIESRHGFNKQTIWMFIRDMIKGTFLSVILGPPIVAAIIFI
AFC1            GVMTWSQITDLPFSLYSTFVIESRHGFNKQTIWMFIRDMIKGTFLSVILGPPIVAAIIFI
BASF_AT1        GVMTWSQITDLPFSLYSTFVIESRHGFNKQTIWMFIRDMIKGTFLSVILGPPIVAAIIFI
PPI-AtCPP       GVMTWSQITDLPFSLYSTFVIESRHGFNKQTIWMFIRDMIKGTFLSVILGPPIVAAIIFI
PPI-BnCPP       GLMTWSQITDLPFSLYSTFVIESRHGFNKQTIWMFIRDMIKGILLSVIPAPPIVAAIIVI
BASF-Corn       GSMVWSQITDLPFSLYSTFVIEARHGFNKQTIWLFIRDMIKGILLSMILGPPIVAAIIYI
                **************:******** *:*:***:** :**:* .******** *


PPI-GmCPP       VQKGGPYLAIYLWVFTFGLSIVMMTLYPVLIAPLFNKFTPLPDGQLREKIEKLASSLNYP
BASF-Gm         VQKGGPYLAIYLWVFTFGLSIVMMTLYPVLIAPLFNKFTPLPDGQLREKIEKLASSLNYP
AF007269        VQKGGPYLAIYLWAFMFILSLVMMTIYPVLIAPLFNKFTPLPDGDLREKIEKLASSLKFP
AT4g-AtCPP      VQKGGPYLAIYLWAFMFILSLVMMTIYPVLIAPLFNKFTPLPDGDLREKIEKLASSLKFP
BASF_AT2        VQKGGPYLAIYLWAFMFILSLVMMTIYPVLIAPLFNKFTPLPDGDLREKIEKLASSLKFP
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AFC1            VQKGGPYLAIYLWAFMFILSLVMMTIYPVLIAPLFNKFTPLPDGDLREKIEKLASSLKFP
BASF_AT1        VQKGGPYLAIYLWAFMFILSLVMMTIYPVLIAPLFNKFTPLPDGDLREKIEKLASSLKFP
PPI-AtCPP       VQKGGPYLAIYLWAFMFILSLVMMTIYPVLIAPLFNKFTPLPDGDLREKIEKLASSLKFP
PPI-BnCPP       VQKGGPYLAIYLWAFMFILSLVMMTIYPVLIAPLFNKFTPLPDGDLREKIEKLASSLKFP
BASF-Corn       VQIGGPYLAIYLWGFMFVLALLMMTIYPIVIAPLFNKFTPLPEGVLREKIEKLAASLKFP
                ** ********** * * *:::***:**::************:* *********:**::*

PPI-GmCPP       LKKLFVVDGSTRSSHSNAYMYGFFKNKRIVPYDTLIQQCKDDEEIVAVIAHELGHWKLNH
BASF-Gm         LKKLFVVDGSTRSSHSNAYMYGFFKNKRIVLYDTLIQQCKDDEEIVAVIAHELGHWKLNH
AF007269        LKKLFVVDGSTRSSHSNAYMYGFFKNKRIVLYDTLIQQCKNEDEIVAVIAHELGHWKLNH
AT4g-AtCPP      LKKLFVVDGSTRSSHSNAYMYGFFKNKRIVLYDTLIQQCKNEDEIVAVIAHELGHWKLNH
BASF_AT2        LKKLFVVDGSTRSSHSNAYMYGFFKNKRIVLYDTLIQQCKNEDEIVAVIAHELGHWKLNH
AFC1            LKKLFVVDGSTRSSHSNAYMYGFFKNKRIVLYDTLIQQCKNEDEIVAVIAHELGHWKLNH
BASF_AT1        LKKLFVVDGSTRSSHSNAYMYGFFKNKRIVLYDTLIQQCKNEDEIVAVIAHELGHWKLNH
PPI-AtCPP       LKKLFVVDGSTRSSHSNAYMYGFFKNKRIVLYDTLIQQCKNEDEIVAVIAHELGHWKLNH
PPI-BnCPP       LKKLFVVDGSTRSSHSNAYMYGFFKNKRIVLYDTLIQQCQNENEIVAVIAHELGHWKLNH
BASF-Corn       LKKLFVVDGSTRSSHSNAYMYGFFKNKRIVLYDTLIQQCSNEDEIVSVIAHELGHWKLNH
                ****************************** ********.:::***:**************

PPI-GmCPP       TVYTFVAMQILTLLQFGGYTLVRNSADLYRSFGFDTQPVLIGLIIFQHTVIPLQQLVSFG
BASF-Gm         TVYTFVAMQILTLLQFGGYTLVRNSADLYRSFGFDTQPVLIGLIIFQHTVIPLQQLVSFG
AF007269        TTYSFIAV-------------------------------------QHTVIPLQHLVSFG
AT4g-AtCPP      TTYSFIAVQILAFLQFGGYTLVRNSTDLFRSFGFDTQPVLIGLIIFQHTVIPLQHLVSFG
BASF_AT2        TTYSFIAVQILAFLQFGGYTLVRNSTDLFRSFGFDTQPVLIGLIIFQHTVIPLQHLVSFG
AFC1            TTYSFIAVQILAFLQFGGYTLVRNSTDLFRSFGFDTQPVLIGLIIFQHTVIPLQHLVSFG
BASF_AT1        TTYSFIAVQILAFLQFGGYTLVRNSTDLFRSFGFDTQPVLIGLIIFQHTVIPLQHPVSFG
PPI-AtCPP       TTYSFIAVQILAFLQFGGYTLLRNSTDLFRSFGFDTQPVLIGLIIFQHTVIPLQHLVSFG
PPI-BnCPP       TTYSFIAVQILAFLQFGGYTLVRNSTDLFRSFGFDTQPVLIGLIIFQHTVIPLQHLVSFD
BASF-Corn       TVYSFVAVQLLMFLQFGGYTLVRSSKDLFGSFGFKDQPVIIGLIIFPHTIIPIQHLLSFR
                *.*:*:*:                                          **:**:*: :**

PPI-GmCPP       LNLVSRSFEFQADGFAKKLGYASGLRG---------------------------------
BASF-Gm         LNLVSRSFEFQADGFAKKLGYASGLRG---------------------------------
AF007269        LNLVSRAFEFQADAFAVKLGYAKDLR-------PALV----KLQVREDNNRTQ-------
AT4g-AtCPP      LNLVSRAFEFQADAFAVKLGYAKDLR-------PALV----KLQVREDNNRTQTVTSICV
BASF_AT2        LNLVSRAFEFQADAFAVKLGYAKDLR-------PALV----KLQE---------------
AFC1            LNLVSRAFEFQADAFAVKLGYAKDLR-------PALVKLQE-------------------
BASF_AT1        LNLVSRAFEFQADAFAVKLGYAKDLRPTLVKLQ---------------------------
PPI-AtCPP       LNLVSRAFEFQADAFAVKLDYAKDLRPALVKLQ---------------------------
PPI-BnCPP       LNLVSRAFEFQADAFAVNLGYAKDLRP---------------------------------
BASF-Corn       LNLVSRAFEFQADAFAKNLGYAPQLR----------------------------------
                ******:******.** :*.**  **

PPI-GmCPP       ------GLVKLQEENLSAMNTDPWYSAYHYSHPPLVERLAALDEPDKKED-
BASF-Gm         ------GLVKLQEENLSAMNTDPCSC-------------------------
AF007269        ----------TEENLSAMNTDPLYSAYHYSHPPLVERLRAIDGEDKKTD-
AT4g-AtCPP      THLNGFFVGILQEENLSAMNTDPLYSAYHYSHPPLVERLRAIDGEDKKTD-
BASF_AT2        ------------ENLSAMNTDPLYSAYHYSHPPLVERLRAIDGEDKKTD-
AFC1            ------------ENLSAMNTDPLHSAYHYSHPPLVERLRAIDGEDKKTD-
BASF_AT1        ------------EENLSAMNTDPLYSAYHYSHPPLVERLRAIDGEDKKTD-
PPI-AtCPP       ------------EENLSTMNTDPLYSAYHYSHPPLVERLRATDGEDKKTD-
PPI-BnCPP       ------ALVKLQEENLSAMNTDPLYSAYHYSHPPLVERLRAIDGEDKKTD-
BASF-Corn       -----AALVKLQEENLSAMNTDPWYSAYHYSHPPLVERLQALEDSDDKKED
                     ****:*****  .
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Example 32: Plant Transformation

Arabidopsis transgenic plants were made by the method of dipping flowering plants into an *Agrobacterium* culture, based on the method of Andrew Bent in, Clough SJ and Bent AF, 1998. Floral dipping: a simplified method for *Agrobacterium*-mediated transformation of

Arabidopsis thaliana. Wild type plants were grown under standard conditions until the plant has both developing flowers and open flowers. The plant was inverted for 2 minutes into a solution of *Agrobacterium* culture carrying the appropriate gene construct. Plants were then left horizontal in a tray and kept covered for two days to maintain humidity and then righted and bagged to continue growth and seed development. Mature seed was bulk harvested.

Transformed T1 plants were selected by germination and growth on MS plates containing 50 μg/ml kanamycin. Green, kanamycin resistant (Kan^R) seedlings were identified after 2 weeks growth and transplanted to soil. Plants were bagged to ensure self fertilization and the T2 seed of each plant harvested separately. During growth of T1 plants leaf samples were harvested, DNA extracted and Southern blot and PCR analysis performed.

T2 seeds were analysed for Kan^R segregation. From those lines that showed a 3:1 resistant phenotype, surviving T2 plants were grown, bagged during seed set, and T3 seed harvested from each line. T3 seed was again used for Kan^R segregation analysis and those lines showing 100% Kan^R phenotype were selected as homozygous lines. Further molecular and physiological analysis was done using T3 seedlings.

Transgenic *Brassica napus, Glycine max* and *Zea maize* plants were produced using *Agrobacterium* mediated transformation of cotyledon petiole tissue. Seeds were sterilized as follows. Seeds were wetted with 95% ethanol for a short period of time such as 15 seconds. Approximately 30 ml of sterilizing solution I was added (70% Javex , 100μl Tween20) and left for approximately 15 minutes. Solution I was removed and replaced with 30 ml of solution II (0.25% mecuric chloride, 100μl Tween20) and incubated for about 10 minutes. Seeds were rinsed with at least 500 ml double distilled sterile water and stored in a sterile dish. Seeds were germinated on plates of $^1/_2$ MS medium, pH 5.8, supplemented with 1% sucrose and 0.7% agar. Fully expanded cotyledons were harvested and placed on Medium I (Murashige minimal organics (MMO), 3% sucrose, 4.5 mg/L benzyl adenine (BA), 0.7% phytoagar, pH5.8). An *Agrobacterium* culture containing the nucleic acid construct of interest was grown for 2 days in AB Minimal media. The cotyledon explants were dipped such that only the cut portion of the petiole is contacted by the *Agrobacterium* solution. The explants were then embedded in Medium I and maintained for 5 days at 24°C, with 16,8 hr light dark cycles.

Explants were transferred to Medium II (Medium I, 300 mg/L timentin,) for a further 7 days and then to Medium III (Medium II, 20 mg/L kanamycin). Any root or shoot tissue which had developed at this time was dissected away. Transfer explants to fresh plates of Medium III

after 14 -21 days. When regenerated shoot tissue developed the regenerated tissue was transferred to Medium IV (MMO, 3% sucrose, 1.0% phytoagar, 300 mg/L timentin, 20 mg/L 20 mg/L kanamycin). Once healthy shoot tissue developed shoot tissue dissected from any callus tissue was dipped in 10X IBA and transferred to Medium V (Murashige and Skooge (MS), 3% sucrose, 0.2 mg/L indole butyric acid (IBA), 0.7% agar, 300 mg/L timentin, 20 mg/L 20 mg/L kanamycin) for rooting. Healthy plantlets were transferred to soil. The above method, with or without modifications, is suitable for the transformation of numerous plant species including *Glycine max, Zea maize* and cotton.

Transgenic *Glycine max, Zea maize* and cotton can be produced using *Agrobacterium*-based methods which are known to one of skill in the art. Alternatively one can use a particle or non-particle biolistic bombardment transformation method. An example of non-particle biolistic transformation is given in U.S. Patent Application 20010026941. This method has been used to produce transgenic *Glycine max* and *Zea maize* plants. Viable plants are propagated and homozygous lines are generated. Plants are tested for the presence of drought tolerance, physiological and biochemical phenotypes as described elsewhere.

The following table identifies the constructs and the species which they have been transformed.

Table 19 Transformation List

SEQ ID NO:	Construct	Species Transformed
99	pBII121-AtCPP	*A. thaliana, B. napus*
100	pBII121-HP-AtCPP	*A. thaliana*
131	pRD29A-AtCPP	*A. thaliana, B. napus*
132	pRD29A-HP-AtCPP	*A. thaliana*
134	MuA-AtCPP	*Glycine max, Zea mays*

Non-limiting examples of vector constructs suitable for plant transformation are given in SEQ ID NO: 99, 5, 35-53.

SEQ ID NO:99

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SEQ ID NO:99 is the nucleic acid sequence of pBI121-AtCPP. Italicized sequences are the right and left border repeats. Underlined sequence is the 35S promoter and bolded sequence is the AtCPP sense sequence.

SEQ ID NO:100

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SEQ ID NO:100 is the nucleic acid sequence of pBI121-HP-AtCPP. Italicized sequences are the right and left border repeats. Underlined sequence is the 35S promoter and bolded sequence is the AtCPP anti-sense sequence. Sequence in upper case is the truncated GUS fragment. Sequence in bold and underlined is the AtCPP sense sequence.

SEQ ID NO:130

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SEQ ID NO:130 is the nucleic acid sequence of pBI121-antisense-AtCPP. Italicized sequences are the right and left border repeats. Underlined sequence is the 35S promoter. Sequence in upper case is the AtCPP anti-sense sequence.

SEQ ID NO:131

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SEQ ID NO:131 is the nucleic acid sequence of RD29A-AtCPP. Italicized sequences are the right and left border repeats. Underlined sequence is the RD29A promoter. Sequence in bold is the AtCPP sense sequence.

SEQ ID NO:132

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gatgggtttttatgattagagtcccgcaattatacatttaatacgcgatagaaaacaaaatata
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cccagtacattaaaaacgtccgcaatgtgttattaagttgtctaagcgtcaatttgtttacacc
acaatatatcctgcca

SEQ ID NO:132 is the nucleic acid sequence of RD29A-HP-AtCPP. Italicized sequences are the right and left border repeats. Underlined sequence is the RD29A promoter. Sequence in bold is the AtCPP anti-sense sequence. Upper case sequence represents the truncated GUS fragment. Bold and underlined sequence represents the *A. thaliana* CaaX prenyl protease sense fragment.

SEQ ID NO:133

gtttacccgccaatatatcctgtcaaacactgatagtttaaactgaaggcgggaaacgacaatc
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GAACATCCATATTGTTTGTTTGTTGAACCCATGCCGAGACTCGATCACGAAAGTTGAGTACAAA
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SEQ ID NO:133 is the nucleic acid sequence of RD29A-antisense-AtCPP. Italicized sequences are the right and left border repeats. Underlined sequence is the RD29A promoter. Sequence in upper case sequence is the AtCPP anti-sense sequence.

SEQ ID NO:134

gtttacccgccaatatatcctgtcaaacactgatagtttaaactgaaggcgggaaacgacaatctgatcatgagcggagaattaagggagtcacgttatgacccccgccgatgacgcgggacaagccgttttacgtttggaactgacagaaccgcaacgttgaaggagccactcagccgcgggtttctggagtttaatgagctaagcacatacgtcagaaaccattattgcgcgttcaaaagtcgcctaaggtcactatcagctagcaaatatttcttgtcaaaaatgctccactgacgttccataaattcccctcggtatccaattagagtctcatattcactctcaatccaaataatctgcaccggatctggatcgtttcgcatgattgaacaagatggattgcacgcaggttctccggccgcttgggtggagaggctattcggctatgactgggcacaacagacaatcggctgctctgatgccgccgtgttccggctgtcagcgcaggggcgcccggttctttttgtcaagaccgacctgtccggtgccctgaatgaactgcaggacgaggcagcgcggctatcgtggctggccacgacgggcgttccttgcgcagctgtgctcgacgttgtcactgaagcgggaagggactggctgctattgggcgaagtgccggggcaggatctcctgtcatctcaccttgctcctgccgagaaagtatccatcatggctgatgcaatgcggcggctgcatacgcttgatccggctacctgcccattcgaccaccaagcgaaacatcgcatcgagcgagcacgtactcggatggaagccggtcttgtcgatcaggatgatctggacgaagagcatcaggggctcgcgccagccgaactgttcgccaggctcaaggcgcgcatgcccgacggcgatgatctcgtcgtgacccatggcgatgcctgcttgccgaatatcatggtggaaaatggccgcttttctggattcatcgactgtggccggctgggtgtggcggaccgctatcaggacatagcgttggctacccgtgatattgctgaagagcttggcggcgaatgggctgaccgcttcctcgtgctttacggtatcgccgctcccgattcgcagcgcatcgccttctatcgccttcttgacgagttcttctgagcgggactctggggttcgaaatgaccgaccaagcgacgcccaacctgccatcacgagatttcgattccaccgccgccttctatgaaaggttgggcttcgga

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CATGAGGAGCACCGTGGAGTAAGAAGACGTTCGAGCCACGTCGAAAAAGCAAGTGTGTTGATGT
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TATAAGAAAGTTAATATCATTTCGAGTGGCCACGCTGAGGGGGATCC**atggcgattcctttcat**
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SEQ ID NO:134 is the nucleic acid sequence of MuA-AtCPP. Italicized sequences are the right and left border repeats. Sequence in upper case is the MuA promoter. The *A. thaliana* CaaX prenyl protease sense sequence is in bold.

SEQ ID NO:135

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CATGAGGAGCACCGTGGAGTAAGAAGACGTTCGAGCCACGTCGAAAAAGCAAGTGTGTTGATGT
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caccacaatatatcctgcca

SEQ ID NO:135 is the nucleic acid sequence of MuA-GmCPP. Italicized sequences are the right and left border repeats. Sequence in upper case is the MuA promoter. The *G. max* CaaX prenyl protease sense sequence is in upper case and bold.

SEQ ID NO:136

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SEQ ID NO:135 is the nucleic acid sequence of pBI121-GmCPP. Italicized sequences are the right and left border repeats. Underlined sequence is the 35S promoter. The *G. max* CaaX prenyl protease sense sequence is in bold.

SEQ ID NO:137

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acaatatatcctgcca

SEQ ID NO:137 is the nucleic acid sequence of pBI121-HP-GmCPP. Italicized sequences are the right and left border repeats. Underlined sequence is the 35S promoter. Bold sequence is the antisense prenyl protease fragment of *G. max*. Bold and underlined sequence is the *G. max* sense prenyl protease fragment and sequence in upper case is the truncated GUS fragment.

SEQ ID NO:138

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SEQ ID NO:138 is the nucleic acid sequence of pBI121-antisense-GmCPP. Italicized sequences are the right and left border repeats. Underlined sequence is the 35S promoter. Sequence in bold is the GmCPP anti-sense sequence.

SEQ ID NO:139

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SEQ ID NO:139 is the nucleic acid sequence of pRD29A-GmCPP. Italicized sequences are the right and left border repeats. Underlined sequence is the RD29A promoter. Sequence in bold is the GmCPP sense sequence.

SEQ ID NO:140

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TGGCAGGAGAAACTGTACACCGACATGTGGAGTGAAGAGTATCAGTGTGCATGGCTGGATATGT
ATCACCGCGTCTTTGATCGCGTCAGCGCCGTCGTCGGTGAACAGGTATGGAATTTCGCCGATTT
TGCGACCTCGCAAGGCATATTGCGCGTTGGCGGTAACAAGAAAGGGATCTTCACTCGCGACCGC
AAACCGAAGTCGGCGGCTTTTCTGCTGCAAAAACGCTGGACTGGCATGAACTTCGGTGAAAAAC
CGCAGCAGGGAGGCAAACAATGAatcaacaactctcctggcgcaccatcgtcggctacagcctc
gggaattgctaccgagctc**acaagatcaagtcacagcaatgcctatatgtatggattcttcaag**
aacaagaggattgtcccttatgacacattaattcaacagtgcaaagacgatgaggaaattgttg
ctgttattgcccatgagttgggacactggaagctcaaccatactgtgtacacatttgttgctat
gcagattcttacacttctacaatttggaggatatacactagtgcgaaattcagctgatctgtat
cgaagctttgggtttgatacgcagccagtcctcattgggctcatcatatttcagcatactgtaa
tcccacttcagcaattggtcagctttggtctgaacctagtcagccgatcatttgaatttcaggc
tgatggctttgccaagaagcttggatatgcatctggattacgcggtggtcttgtgaaactacag
gaggagaatctgtcagctatgaatacagatccttggtactctgcttatcactattctcatcctc
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atttggcaataaagtttcttaagattgaatcctgttgccggtcttgcgatgattatcatataat
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ggtttttatgattagagtcccgcaattatacatttaatacgcgatagaaaacaaaatatagcgc
gcaaactaggataaattatcgcgcgcggtgtcatctatgttactagatcgggaattcactggcc
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ctcgaccccaaaaaacttgatttgggtgatggttcacgtagtgggccatcgccctgatagacgg
tttttcgccctttgacgttggagtccacgttctttaatagtggactcttgttccaaactggaac
aacactcaaccctatctcgggctattcttttgatttataagggattttgccgatttcggaacca
ccatcaaacaggattttcgcctgctggggcaaaccagcgtggaccgcttgctgcaactctctca
gggccaggcggtgaagggcaatcagctgttgcccgtctcactggtgaaaagaaaaaccacccca

gtacattaaaaacgtccgcaatgtgttattaagttgtctaagcgtcaattt*gtttacaccacaa tatatcctgcca*

SEQ ID NO:140 is the nucleic acid sequence of pRD29A-HP-GmCPP. Italicized sequences are the right and left border repeats. Underlined sequence is the RD29A promoter. Sequence in bold is the GmCPP antisense sequence, bold and underlined sequence is the GmCPP sense sequence.

SEQ ID NO:141

gtttacccgccaatatatcctgtcaaacactgatagtttaaactgaaggcgggaaacgacaatctgatcatgagcggagaattaagg
gagtcacgttatgacccccgccgatgacgcgggacaagccgttttacgtttggaactgacagaaccgcaacgttgaaggagccac
tcagccgcgggtttctggagtttaatgagctaagcacatacgtcagaaaccattattgcgcgttcaaaagtcgcctaaggtcactat
cagctagcaaatatttcttgtcaaaaatgctccactgacgttccataaattcccctcggtatccaattagagtctcatattcactctca
atccaaataatctgcaccggatctggatcgtttcgcatgattgaacaagatggattgcacgcaggttctccggccgcttgggtggag
aggctattcggctatgactgggcacaacagacaatcggctgctctgatgccgccgtgttccggctgtcagcgcaggggcgcccggt
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ttccttgcgcagctgtgctcgacgttgtcactgaagcgggaagggactggctgctattgggcgaagtgccggggcaggatctcctgt
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cgaccaccaagcgaaacatcgcatcgagcgagcacgtactcggatggaagccggtcttgtcgatcaggatgatctggacgaaga
gcatcaggggctcgcgccagccgaactgttcgccaggctcaaggcgcgcatgcccgacggcgatgatctcgtcgtgacccatggc
gatgcctgcttgccgaatatcatggtggaaaatggccgcttttctggattcatcgactgtggccggctgggtgtggcggaccgctatc
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ctcccgattcgcagcgcatcgccttctatcgccttcttgacgagttcttctgagcgggactctggggttcgaaatgaccgaccaagcg
acgcccaacctgccatcacgagatttcgattccaccgccgccttctatgaaaggttgggcttcggaatcgttttccgggacgccggc
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cattacgacagcaacggccgacaagcacaacgccacgatcctgagcgacaatatgatcgggcccggcgtccacatcaacggcgt
cggcggcgactgcccaggcaagaccgagatgcaccgcgatatcttgctgcgttcggatattttcgtggagttcccgccacagaccc
ggatgatccccgatcgttcaaacatttggcaataaagtttcttaagattgaatcctgttgccggtcttgcgatgattatcatataatttc
tgttgaattacgttaagcatgtaataattaacatgtaatgcatgacgttatttatgagatgggtttttatgattagagtcccgcaattat
acatttaatacgcgatagaaaacaaaatatagcgcgcaaactaggataaattatcgcgcgcggtgtcatctatgttactagatcgg
gcctcctgtcaatgctggcggcggctctggtggtggttctggtggcggctctgagggtggtggctctgagggtggcggttctgaggg
tggcggctctgagggaggcggttccggtggtggctctggttccggtgattttgattatgaaaagatggcaaacgctaataaggggg
ctatgaccgaaaatgccgatgaaaacgcgctacagtctgacgctaaaggcaaacttgattctgtcgctactgattacggtgctgcta
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gcagtgagcgcaacgcaattaatgtgagttagctcactcattaggcaccccaggctttacactttatgcttccggctcgtatgttgtgt
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aacaaacttacgaaatttaggtagaacttatatacattatattgtaatttttgtaacaaaatgttttttattattattatagaatttttactg
gttaaattaaaaatgaatagaaaaggtgaattaagaggagagaggaggtaaacattttcttctatttttttcatattttcaggataaat
tattgtaaaagtttacaagatttccatttgactagtgtaaatgaggaatattctctagtaagatcattatttcatctacttcttttatcttc
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aataaaagatcatacctattagaacgattaaggagaaatacaattcgaatgagaaggatgtgccgtttgttataataaacagccac
acgacgtaaacgtaaaatgaccacatgatgggccaatagacatggaccgactactaataatagtaagttacatttaggatggaa
taaatatcataccgacatcagttttgaaagaaaagggaaaaaaagaaaaaataaataaaagatatactaccgacatgagttcca

aaaagcaaaaaaaaaagatcaagccgacacagacacgcgtagagagcaaaatgactttgacgtcacaccacgaaaacagacgc
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aatcatcaggaataaagggtttgattacttctattggaaaggactctagaggatccccgggttagtcttccttcttatccggttcgtccag
cgcggccaatctttcaacaaggggaggatgagaatagtgataagcagagtaccaaggatctgtattcatagctgacagattctcctcctgta
gtttcacaagaccaccgcgtaatccagatgcatatccaagcttcttggcaaagccatcagcctgaaattcaaatgatcggctgactaggttca
gaccaaagctgaccaattgctgaagtgggattacagtatgctgaaatatgatgagcccaatgaggactggctgcgtatcaaacccaaagctt
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gagcttccagtgtcccaactcatgggcaataacagcaacaatttcctcatcgtctttgcactgttgaattaatgtgtcataagggacaatcctctt
gttcttgaagaatccatacatataggcattgctgtgacttgatcttgtggatccatcgacaacaaatagtttctttaacggatagttgagggagg
aagcaagtttctcgattttctccctgagttgaccatctggaagtggagtgaacttattgaagagtggagctattagtactggataaagggtcatc
atcacaatagaaagaccaaacgtaaaaacccaaagatagatggccaagtatggacctcctttctgtactattacaatgattgcagccacaata
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tcacaaaagttgagtacagagaaaagggcaaatctgttatctgtgaccaaatcatcagccctgctaagaaggcaagggtatgcagtatttcat
tctcagcattgaaaccagctattgtcataaaatctcctgatttcttccaaaaccagggcaataccccaaagtacaaaattgtagagtctgtcact
attgtcacaaactcgtgaacaaaatggaagtggcttttatcaagactataggctctagatttctcaaatttctcttggctgataacaccctctaaa
gtctttggaagagtaggaagtttgagggccctatgttgtcgcacatccaagtaagtttcaaaaatgtacattaatatcataaatccgacaacgg
cttccatgtagggaaacgccat**gagctcgaatttccccgatcgttcaaacatttggcaataaagtttcttaagattgaatcctgttgccg**
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ccgcttgctgcaactctctcagggccaggcggtgaagggcaatcagctgttgcccgtctcactggtgaaaagaaaaaccacccca
gtacattaaaaacgtccgcaatgtgttattaagttgtctaagcgtcaattt*gtttacaccacaatatatcctgcca*

SEQ ID NO:141 is the nucleic acid sequence of pRD29A-antisense-GmCPP. Italicized sequences are the right and left border repeats. Underlined sequence is the RD29A promoter. Sequence in bold is the GmCPP antisense sequence.

SEQ ID NO:142

*gtttacccgccaatatatcctgt*caaacactgatagtttaaactgaaggcgggaaacgacaatc
tgatcatgagcggagaattaagggagtcacgttatgacccccgccgatgacgcgggacaagccg
ttttacgtttggaactgacagaaccgcaacgttgaaggagccactcagccgcgggtttctggag
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tccaattagagtctcatattcactctcaatccaaataatctgcaccggatctggatcgtttcgc
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atgactgggcacaacagacaatcggctgctctgatgccgccgtgttccggctgtcagcgcaggg
gcgcccggttctttttgtcaagaccgacctgtccggtgccctgaatgaactgcaggacgaggca
gcgcggctatcgtggctggccacgacgggcgttccttgcgcagctgtgctcgacgttgtcactg

aagcgggaagggactggctgctattgggcgaagtgccggggcaggatctcctgtcatctcacct
tgctcctgccgagaaagtatccatcatggctgatgcaatgcggcggctgcatacgcttgatccg
gctacctgcccattcgaccaccaagcgaaacatcgcatcgagcgagcacgtactcggatggaag
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tatcgccttcttgacgagttcttctgagcgggactctggggttcgaaatgaccgaccaagcgac
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gcgcgcggtgtcatctatgttactagatcgggcctcctgtcaatgctggcggcggctctggtgg
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aaagattcaggactaactgcatcaagaacacagagaaagatatatttctcaagatcagaagtac
tattccagtatggacgattcaaggcttgcttcacaaaccaaggcaagtaatagagattggagtc
tctaaaaaggtagttcccactgaatcaaaggccatggagtcaaagattcaaatagaggacctaa

cagaactcgccgtaaagactggcgaacagttcatacagagtctcttacgactcaatgacaagaa
gaaaatcttcgtcaacatggtggagcacgacacacttgtctactccaaaaatatcaaagataca
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caaatgccatcattgcgataaaggaaaggccatcgttgaagatgcctctgccgacagtggtccc
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agcaagtggattgatgtgatatctccactgacgtaagggatgacgcacaatcccactatccttc
gcaagacccttcctctatataaggaagttcatttcatttggagagaacacgggggactctagag
gatcc**atggcgattcctttcatggaaaccgtcgttggttttatgatagtgatgtacgtttttga**
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gccggtcttgcgatgattatcatataatttctgttgaattacgttaagcatgtaataattaaca
tgtaatgcatgacgttatttatgagatgggtttttatgattagagtcccgcaattatacattta
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gttacccaacttaatcgccttgcagcacatccccctttcgccagctggcgtaatagcgaagagg

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ctcactggtgaaaagaaaaaccaccccagtacattaaaaacgtccgcaatgtgttattaagttg
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SEQ ID NO:142 is the nucleic acid sequence of pBI121-BnCPP. Italicized sequences are the right and left border repeats. Underlined sequence is the 35S promoter. Sequence in bold is the BnCPP antisense sequence.

SEQ ID NO:143

gtttacccgccaatatatcctgtcaaacactgatagtttaaactgaaggcgggaaacgacaatc
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aagattgaatcctgttgccggtcttgcgatgattatcatataatttctgttgaattacgttaag
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GCGAACAGTTCCTGATTAACCACAAACCGTTCTACTTTACTGGCTTTGGTCGTCATGAAGATGC
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ATGAACATGGCATCGTGGTGATTGATGAAACTGCTGCTGTCGGCTTTTCGCTCTCTTTAGGCAT
TGGTTTCGAAGCGGGCAACAAGCCGAAAGAACTGTACAGCGAAGAGGCAGTCAACGGGGAAACT
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ACTGGCGGAAGCAACGCGTAAACTCGACCCGACGCGTCCGATCACCTGCGTCAATGTAATGTTC
TGCGACGCTCACACCGATACCATCAGCGATCTCTTTGATGTGCTGTGCCTGAACCGTTATTACG
GATGGTATGTCCAAAGCGGCGATTTGGAAACGGCAGAGAAGGTACTGGAAAAAGAACTTCTGGC
CTGGCAGGAGAAACTGTACACCGACATGTGGAGTGAAGAGTATCAGTGTGCATGGCTGGATATG
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TTGCGACCTCGCAAGGCATATTGCGCGTTGGCGGTAACAAGAAAGGGATCTTCACTCGCGACCG
CAAACCGAAGTCGGCGGCTTTTCTGCTGCAAAAACGCTGGACTGGCATGAACTTCGGTGAAAAA
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acattaaaaacgtccgcaatgtgttattaagttgtctaagcgtcaatttgtttacaccacaata
tatcctgcca

SEQ ID NO:143 is the nucleic acid sequence of pBI121-HP-BnCPP. Italicized sequences are the right and left border repeats. Underlined sequence is the 35S promoter. Sequence in bold is the BnCPP antisense sequence, bold and underlined sequence is the BnCPP sense fragment and upper case indicates the truncated GUS fragment.

SEQ ID NO:144

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ctcactggtgaaaagaaaaaccaccccagtacattaaaaacgtccgcaatgtgttattaagttg
tctaagcgtcaattt*gtttacaccacaatatatcctgcca*

SEQ ID NO:144 is the nucleic acid sequence of pBI121-antisense-BnCPP. Italicized sequences are the right and left border repeats. Underlined sequence is the 35S promoter. Sequence in bold is the BnCPP antisense sequence.

SEQ ID NO:145

*gtttacccgccaatatatcctgtc*aaacactgatagtttaaactgaaggcgggaaacgacaatc
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agcgtcaatttgtttacaccacaatatatcctgcca

SEQ ID NO:145 is the nucleic acid sequence of pRD29A-BnCPP. Italicized sequences are the right and left border repeats. Underlined sequence is the RD29A promoter. Sequence in bold is the BnCPP sense sequence.

SEQ ID NO:146

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GCGGAAGCAACGCGTAAACTCGACCCGACGCGTCCGATCACCTGCGTCAATGTAATGTTCTGCG
ACGCTCACACCGATACCATCAGCGATCTCTTTGATGTGCTGTGCCTGAACCGTTATTACGGATG
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CAGGAGAAACTGTACACCGACATGTGGAGTGAAGAGTATCAGTGTGCATGGCTGGATATGTATC
ACCGCGTCTTTGATCGCGTCAGCGCCGTCGTCGGTGAACAGGTATGGAATTTCGCCGATTTTGC
GACCTCGCAAGGCATATTGCGCGTTGGCGGTAACAAGAAAGGGATCTTCACTCGCGACCGCAAA
CCGAAGTCGGCGGCTTTTCTGCTGCAAAAACGCTGGACTGGCATGAACTTCGGTGAAAAACCGC
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ctgcca

SEQ ID NO:146 is the nucleic acid sequence of pRD29A-HP-BnCPP. Italicized sequences are the right and left border repeats. Underlined sequence is the RD29A promoter. Sequence in bold is the BnCPP antisense sequence, bold and underlined sequence is BnCPP sense fragment and the upper case sequence represents the truncated GUS fragment.

SEQ ID NO:147

*gtttacccgccaatatatcctgtc*aaacactgatagtttaaactgaaggcgggaaacgacaatc
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agcgtcaatttgtttacaccacaatatatcctgcca

SEQ ID NO:147 is the nucleic acid sequence of pRD29A-antisense-BnCPP. Italicized sequences are the right and left border repeats. Underlined sequence is the RD29A promoter. Sequence in bold is the BnCPP antisense sequence.

SEQ ID NO:148

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ggcggcgactgcccaggcaagaccgagatgcaccgcgatatcttgctgcgttcggatattttcg
tggagttcccgccacagacccggatgatccccgatcgttcaaacatttggcaataaagtttctt
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catgtaataattaacatgtaatgcatgacgttatttatgagatgggtttttatgattagagtcc
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cgccggctttccccgtcaagctctaaatcgggggctcccttagggttccgatttagtgcttta
cggcacctcgaccccaaaaaacttgatttgggtgatggttcacgtagtgggccatcgccctgat
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tggaacaacactcaaccctatctcgggctattcttttgatttataagggattttgccgatttcg
gaaccaccatcaaacaggattttcgcctgctggggcaaaccagcgtggaccgcttgctgcaact
ctctcagggccaggcggtgaagggcaatcagctgttgcccgtctcactggtgaaaagaaaaacc
accccagtacattaaaaacgtccgcaatgtgttattaagttgtctaagcgtcaatttgtttaca
ccacaatatatcctgcca

SEQ ID NO:148 is the nucleic acid sequence of MuA-BnCPP. Italicized sequences are the right and left border repeats. Underlined sequence is the MuA promoter. Sequence in bold is the BnCPP sense sequence.

Example 33. Southern Analysis

Genomic Southern blot analysis of transgenic *Arabidopsis* was performed using standard techniques known to one skilled in the art. Typically, 10μg of DNA was electrophoresed in a 0.8% agarose gel and transferred to an appropriate membrane such as Hybond N+ (Amersham Pharmacia Biotech). Pre-hybridization and hybridization conditions were as suggested by the membrane manufacturer, typically at 65°C. The final stringency wash was typically at 1XSSC and 0.1% SDS at 65°C. The NPTII coding region was typically used as the radiolabeled probe in Southern blot analysis.

Thirty-seven *Arabidopsis* lines were selected as homozygous pBI121-AtCPP over-expression lines for further examination. Figure 27 shows a representative blot confirming the presence of the pBI121-AtCPP transgene. Lines were confirmed to be transgenic by PCR analysis using transgene specific primers in the PCR assays.

Thirty-three *Arabidopsis* lines were selected as homozygous pBI121-HP-AtCPP hair-pin down-regulation lines for further examination. Figure 28 shows a representative blot confirming the presence of the pBI121-HP-AtCPP hair-pin construct. All lines were confirmed to be transgenic by PCR analysis using transgene specific primers in the PCR assays.

Arabidopsis lines were selected as homozygous pRD29A-AtCPP over-expression lines for further examination. Figure 29 shows a representative blot confirming the presence of the pRD29A-AtCPP transgene. Lines were confirmed to be transgenic by PCR analysis using transgene specific primers in the PCR assays.

Arabidopsis lines were selected as homozygous pRD29A-HP-AtCPP lines for further examination. Figure 30 shows a representative blot confirming the presence of the pRD29A-HP-AtCPP transgene. Lines were confirmed to be transgenic by PCR analysis using transgene specific primers in the PCR assays.

Example 34: PCR analysis of transgenic plants

PCR was used as a method to confirm the presence of the transgene in all transgenic lines and every construct.. Typical PCR mixtures contained: 1X reaction buffer (10mM Tris-HCl pH 8.8, 1.5mM $MgCl_2$,, 50mM KCl), dNTP's at 200μM, 1pM forward and reverse primer, 2.5U. *Taq* DNA polymerase, and template plus water to a final volume of 50μL. Reactions were run at 1 minute 94°C, 1 minute 60°C, 1 minute 72°C, for 30 cycles. Primers used in the analysis of pBI121-AtCPP and pBI121-HP-AtCPP transgenic plants were as shown in Table 20. Primers used in the analysis of pRD29A-AtCPP were RD29AP1 (SEQ ID NO:161) and SEQ ID NO:102. Primers used in the analysis of pRD29A-HP-AtCPP transgenic plants were those identified as RD29AP1 (SEQ ID NO:161), SEQ ID NO:103 and SEQ ID NO:103, Nosterm-RV (SEQ ID NO:162).

Table 20.

pBI121-AtCPP BamFW: 5'-GCCGACAGTGGTCCCAAAGATGG-3'
(SEQ ID NO:105)

p35S-AtCPP SmaRV: 5'-AAACCCGGGTTAATCTGTCTTCTTGTCTTCTCCA-3' (SEQ ID NO:102)

p35S-HP-AtCPP BamFW: 5'-CTGGAGCTCTTTTACCGAGGTTGGGCCTTGATCC-3' (SEQ ID NO:103)

p35S-HP-AtCPP SmaRV: 5'-GCAAGACCGGCAACAGGA-3'
(SEQ ID NO:108)

pRD29AP1: 5'-TTTAAGCTTGGAGCCATAGATGCAATTCAA -3'
(SEQ ID NO:161)

pRD29AP1: 5'-TTTAAGCTTGGAGCCATAGATGCAATTCAA -3'
(SEQ ID NO:161)

Nosterm-RV: 5'-GCAAGACCGGCAACAGGA-3'
(SEQ ID NO:162)

Example 35: Northern analysis of transgenic plants

Total RNA was isolated from developing leaf tissue of 27 35S-AtCPP *Arabidopsis* lines (T3 plants). Approximately 10 μg of total RNA was loaded into each lane. The Northern blot was first probed with P^{32} labeled, single-stranded antisense transcript of AtCPP which detects sense transcript, then stripped and re-probed with cDNA of β-tubulin that was used as a reference. The hybridizing bands of AtCPP and β-tubulin were scanned and quantified using the UN-Scan-It programme (Silk Scientific, Utah, USA), and the ratio of the two hybridizing bands for each sample was obtained. The ratio of the wild type plants was set to 100%, and was compared with those of the transgenic lines. Twenty-one out of twenty-seven lines showed higher expression of AtCPP transcript as compared to the wild type. Values ranged from 104 % to 282 % of wild type. The results of five lines (35, 84, 76, 136, and 156) of the 21 over-expressing lines is shown in Figure 31.

Example 36: Production of polyclonal antibodies against AtCPP

Anti-AtCPP antibodies were generated using AtCPP fusion protein over-expressed in *E. coli*. The over-expression vector, pMAL-p2, contains 1175 bp malE gene that is located upstream of AtCPP and encodes a 43 KDa maltose-binding protein (MBP). The 1275 bp *Bam*HI/*Sma*I DNA fragment of AtCPP was inserted into pMAL-p2 at *Bam*HI and *Sal*I sites. The *Sal*I site was converted into blunt end using Klenow fragment. The resulting fusion protein

MBP-AtCPP was then over-expressed in DH5α, and purified by one-step affinity for MBP as described by the manufacturer (New England Biolab). The soluble fraction of the crude bacterial extract containing the MBP-AtCPP fusion protein was loaded to a amylose column (1.5 cm x 10.0 cm), and the proteins were eluted with 10 mM maltose in column buffer (50 mM Tris-HCl, pH 7.5, 1 mM EDTA, and 200 mM NaCl). Fractions containing purified MBP-AtCPP fusion protein were pooled, and concentrated with a Centriprep-30 concentrator (Amicon). All purification steps were carried out at 4°C. To generate an antibody, the purified fusion protein was further separated by SDS-PAGE and the Coomassie stained band corresponding to the fusion protein was excised. The identity of the fusion protein was confirmed by Western analysis using anti-MBP antibodies (purchased from New England Biolab). The protein was eluted from the gel slice by electroelution and then emulsified in Ribi adjuvant (Ribi Immunochem) to a final volume of 1 ml. MBP-AtCPP protein was injected into a 3 kg New Zealand rabbit on day 1 and booster injections were given on day 21 and day 35 with 175 μg of the protein each time. High-titer antisera were obtained one week after the final injection.

Example 37: Western blot analysis of 35S-AtCPP transgenic lines using Anti-AtCPP antibodies.

Western analysis was performed to examine expression level of AtCPP in the transgenic lines compared with that of wild type plants. Anti-Bip antibody, an ER lumenal protein (Stressgen, Victoria, BC, Canada) was used as a reference. Total proteins were extracted from developing leaf tissue of five ABA^S lines and a wild type control.. The antigenic protein bands of AtCPP and Bip were scanned and quantified using the UN-Scan-It programme (Silk Scientific, Utah, USA) and the ratio of the two protein bands for each sample was obtained. The ratio of the wild type plants was set to 100%, and was compared with those of the transgenic lines. Data is presented in Figure 31 indicating that the AtCPP protein level was increased in the transgenic lines compared to the wild type plants.

Example 38: ABA sensitivity of transgenic seedlings.

Approximately 100 seeds were assessed per line per 9 cm plate. Seeds were plated on minimal medium (1/2 MS) supplemented with no ABA or 1.0 μM ABA. Plates were chilled for 3 days at 4 ^{0}C in the dark, and incubated for up to 21 days at 22 ^{0}C with 24 hour continuous light. Plates were assessed for germination, cotyledon expansion, true leaf development and seedling vigor. Seedlings were assessed for ABA sensitivity over 21 days of growth at which time sensitive seedlings were arrested at the cotyledon stage, lacked true leaves, and showed

inhibition of root growth. Wild type control Columbia plants had two to three pairs of true leaves and a well developed root system. Lines were categorized as ABA sensitive (ABA^S) if less than 1% of plants looked like control, moderately ABA sensitive (ABA^{MS}) if more than 1% but less than 50% of looked like control, or ABA insensitive (ABA^{Wt}) if greater than 50% looked like control.

For example, if a plate had 20 healthy seedlings and the control plate had 60 healthy seedlings, the line would be 33% of control and categorized as moderately ABA sensitive.

All four vector constructs (pBI121-AtCPP, pBI121Hp-AtCPP, pRD29AHp-AtCPP, pRD29A-ATCPP) have resulted in transgenic lines of *Arabidopsis* which have increased sensitivity to ABA which is indicative of stress tolerance. The data for all 4 constructs is shown in Figure 32. Of the lines transformed with the pBI121-AtCPP construct to over-express the AtCPP gene, 58% (21 out of 36) were classified as sensitive and an added 30% (11 out of 36) were classified as moderately sensitive. These lines were tested again in T4 and T5 generations and their ABA sensitivity was still present indicating that ABA sensitivity is an inheritable trait. Of the lines transformed with the pBI121-HP-AtCPP construct to down-regulate the AtCPP gene by double stranded RNA-inhibition, 15% (7 out of 45) were classified as sensitive and 31% (14 out of 45) were classified as moderately sensitive. To illustrate the increased sensitivity of transgenic lines to ABA, Figure 33 shows the results of germination and seedling development over a range of ABA concentrations. Wild type and pRD29A-HP-AtCPP are compared. Of the lines transformed with pRD29AHp-AtCPP 70% (12 out of 17) showed high sensitivity and 24% (4 out of 17) showed moderate sensitivity to ABA. Of the lines transformed with pRD29A-AtCPP 29% (5 out of 17) showed high sensitivity and 12% (2 out of 17) moderate sensitivity to ABA. Clearly all 4 transgene constructs are altering ABA sensitivity and ABA signal transduction.

Example 39: Drought Experiments

Arabidopsis plants were grown five plants per 4" or 3" pot, in a replicated water-stress experiment. All pots were filled with equal amounts of homogeneous premixed and wetted soil. Plants were grown under 16 hour daylight (150-200 $\mu mol/m^2/s$) at 22 °C and 70% relative humidity. On the day that the first flower opened drought treatment was initiated. First soil water content in each pot was equalized on a weight basis and any further watering of plants was stopped. Daily measurements of soil water content were taken by recording total pot weight. At the end of the drought treatment (6 to 9 days for experiments in 4" pots and 4-5 days for

experiments in 3" pots) plants were harvested and shoot dry weights determined. Differences in plant growth were factored into the analysis by expressing water loss on a per gram shoot dry weight basis.

39a) **pBI121-AtCPP, Drought stress screen:**

Analysis of pBI121-AtCPP transgenic lines during water-stress treatment experiments of up to an eight day period, shows a strong trend towards increased soil water content and reduced water loss per gram of shoot biomass. After three days of water-stress treatment most lines had increased soil water content relative to the wild type control with four out of twenty-four lines, 146, 149, 156 and 97, showing a statistically significant difference. The amount of water lost per gram of shoot biomass was lower for all lines except one (95), and thirteen of these lines were significantly different from the wild type Columbia control (Figure 34). All of the lines showing a statistically significant lower water loss per gram shoot biomass also showed an increased ABA sensitivity. There is also a strong trend, for all but one line (95), which is ABA^{Wt}, towards greater shoot biomass at the end of the drought stress treatment. Seven of those lines 136, 146, 23, 46, 76, 84 and 9, were statistically significant from control at a p=0.05 value.

39b) **pBI121-AtCPP, Water loss per gram shoot biomass during water stress treatment:**

Lines 35, 76, 95 and a wild type control were grown and placed under a water-stress treatment as above. Plants were harvested at 2 days, 4 days and 6 days of drought treatment. The ABA^{S} lines, 35 and 76, showed a statistically significant reduction in water-loss relative to shoot dry weight at all three time points (Table 21). Additionally, the two ABA^{S} transgenic lines had increased shoot biomass, due to increased leaf biomass, and maintained higher soil water contents during drought treatment.

Table 21. Water loss (g) per Shoot dry weight (g) after 2, 4 and 6 days of drought-stress treatment. Values in bold indicate statistically significant differences from Columbia.

	2 days		4 days		6 days	
Line	Mean	Std. Error	Mean	Std. Error	Mean	Std. Error
35	**212.5**	3.5	**308.0**	9.9	**297.7**	11.2
76	**227.2**	5.8	**321.2**	8.5	**293.8**	5.0
95	287.0	5.1	**377.3**	14.8	348.5	25.5
Columbia	265.3	11.8	408.2	7.7	345.9	6.7

Wild type						

39c) **pBI121-AtCPP, Drought stress and shoot recovery:**

Water-stress tolerance and determination of post drought-treatment recovery ability was assessed using 20 of the 24 pBI121-AtCPP transgenic lines. Drought treatment was imposed for 6 days after which the plants were watered and allowed to grow for 6 days. Recovered shoot fresh biomass was then determined. Soil water content of these plants was measured daily during the drought treatment and the results confirm previously seen trends. All ABA sensitive (ABA^S) lines that showed a statistically significantly reduction of water loss on a per gram dry weight basis in experiment 39a, continued to show a significant greater soil water content than control plants in this experiment (Table 22). Additionally, Table 22 shows that the recovered shoot fresh biomass after 6 days of drought treatment was significantly greater in all the ABAs lines than Columbia.

Table22. Soil water content on day 3 of drought treatment and recovered shoot fresh weight after 6 days of drought treatment (values in bold were significantly different from Columbia at p=0.05)

	ABA status	soil water content day 3		recovered shoot biomass	
Line	ABA	Mean (% initial)	Std Error	Mean (g)	Std Error
136	ABA^S	**46.6**	1.9	**4.5**	0.16
14	ABA^S	**50.25**	0.7	**4.1**	0.12
146	ABA^S	**45.9**	2.5	**4.0**	0.11
147	ABA^S	45.1	1.7	**4.0**	0.15
149	ABA^S	45.3	1.8	**3.8**	0.17
156	ABA^S	**47.1**	1.9	**4.0**	0.134
23	ABA^S	**49**	1.4	**4.0**	0.17
33	ABA^S	**46.9**	1.6	**4.3**	0.14
35	ABA^S	41.7	1.7	**4.0**	0.11

46	ABA^S	44.8	1.7	**3.8**	0.09
63	ABA^S	**46.3**	1.4	**4.0**	0.19
76	ABA^S	**47.8**	1.0	**3.9**	0.17
79	ABA^S	45.4	1.1	**4.1**	0.09
84	ABA^S	**46.8**	1.9	**4.1**	0.16
85	ABA^S	45.3	1.9	**4.0**	0.12
9	ABA^S	45.2	2.1	**3.9**	0.12
93	ABA^{wt}	43.5	1.2	2.8	0.07
94	ABA^S	**46.9**	1.5	**3.9**	0.13
97	ABA^S	**53**	1.2	**3.8**	0.16
95	ABA^{Wt}	41.9	1.2	2.7	0.06
Columbia	ABA^{Wt}	41.3	1.0	2.7	0.04

39d) **pBI121-AtCPP, Seed yield after drought stress treatment:**

Seed yield after drought stress during flowering was examined using ten pBI121-AtCPP transgenic lines, eight of which were ABA^S. Plants were grown one per 4" pot and were exposed to 9 days of drought treatment as described above. A second group of plants was grown and maintained under well watered conditions as the optimal group. After 9 days of drought treatment plants were re-watered and allowed to continue growth and seed set to maturity. After drought-treatment conditions all eight ABA^S lines had increased yields relative to controls, which ranged from 109% to 126% of the Columbia (Table 23). Drought-treatment resulted in a reduction of yield in all lines, including controls, relative to plants grown under optimal conditions. Expression of the seed yields obtained from drought-treated group relative to the same line under optimal conditions shows that the transgenics preserve a larger percentage of optimal seed yield than do wild type lines.

Table 23. Seed Yield following 9 days drought-treatment

	ABA status	Seed Yield (g per plant)			
Line	ABA	Mean (g)	Std Error	% Columbia	% Optimal
156	ABA^S	0.735	0.044	126.2	83.7
63	ABA^S	0.675	0.061	116.0	71.0
146	ABA^S	0.666	0.053	114.4	72.9
94	ABA^S	0.644	0.052	110.6	68.8
84	ABA^S	0.642	0.049	110.4	61.8
76	ABA^S	0.631	0.055	108.5	66.6
136	ABA^S	0.630	0.051	108.3	74.1
35	ABA^S	0.614	0.054	105.6	74.2
93	ABA^{Wt}	0.567	0.041	97.5	60.0
95	ABA^{Wt}	0.388	0.088	66.7	43.4
Columbia	ABA^{Wt}	0.582	0.060	100	53.8

39e) **pBI121-AtCPP, Seed yield and growth under optimal water conditions:**

The lines evaluated above and a number of additional lines were examined in a growth and yield experiment under optimal, well-watered conditions. Results indicated that the ABA^S lines were shorter at the stage of first open flower, had more rosette leaves, however, by maturity there were no differences in plant height of transgenics and Columbia. Moreover, the ABA^S transgenics showed similar or higher seed yields ranging from 95% to 121% of the wild type control (Figure 35).

39g) **pRD29A-HP-AtCPP screen for drought tolerant phenotype:**

Analysis of 17 transgenic lines identified 7 candidate drought tolerant lines (12, 22, 23, 47, 82, 83, 90) on the basis of higher soil water content and lower water loss per g of shoot dry weight (Table24). All 7 drought tolerant candidate lines showed strong ABA sensitivity and lines that did not show drought tolerance did not show ABA sensitivity.

Table 24. Soil water content after 3 days of drought treatment and water lost per g shoot dry weight. Values in bold are statistically different from those of Columbia wild type (p=0.05)

	ABA status	soil water content day 2		water lost in 2days/g shootDW	
Line	ABA	Mean (% initial)	Std Error	Mean (g/g)	Std Error
10	ABA^{S}	33.4	1.6	199.1	4.5
11	ABA^{S}	34.6	3.3	**173.1**	1.6
12	ABA^{S}	36.2	2.0	179.5	5.0
126	ABA^{MS}	32.5	2.6	199.1	4.1
127	ABA^{MS}	33.5	2.0	195.6	10.6
14	ABA^{S}	32.7	1.2	203	4.9
17	ABA^{S}	29.9	1.8	200.7	7.3
22	ABA^{S}	**39.3**	2.1	**170.0**	3.0
23	ABA^{S}	35.7	1.4	**174.9**	2.6
42	ABA^{MS}	28	0.7	185.4	5.8
47	ABA^{S}	35.9	2.2	181.2	7.7
7	ABA^{Wt}	35	1.3	201.8	5.1
82	ABA^{S}	36.7	2.2	178.3	4.0
83	ABA^{S}	**40**	1.4	180.7	6.9
9	ABA^{S}	31.4	1.4	173.8	8.7
90	ABA^{S}	**38.2**	1.3	**177.6**	6.2
93	ABA^{Wt}	30.7	1.8	175.3	4.6
Columbia	ABA^{Wt}	32.1	1.2	196.9	6.2

Example 40. Growth Analysis

The growth analysis of most promising constructs has been set up at 3 stages. Eight plants per line were grown in 3" pots with one plant per pot at 22C, 16hr light (150-200 $\mu mol/m^2/s$) and 70% RH. Plants were harvested at vegetative growth stage (2 week old seedlings), bolting growth stage (at first open flower) and mid-flowering growth stage (5 to 7 days from first open flower). Also, in some growth experiments additional group of plants was grown in 4" pots (one per pot and 10 plants per line) to maturity for seed yield determinations.

40a) **pBI121-AtCPP growth under optimal and biotic stress conditions**

The growth and productivity of pBI121-AtCPP transgenic *Arabidopsis* lines was examined at several stages of development under optimal growth conditions. Although optimal growth conditions were maintained, plants were assessed to be under a degree of stress that was later determined to be a result of the soil properties. Soil analysis found a fungal contaminant that was believed to be responsible for the biotic stress. This stress could be negated by sterilization of the soil prior to use. Eight ABA^S lines, two with normal ABA sensitivity (ABA^{Wt}) and a wild type Columbia control were analyzed.

Figure 36 presents the results of various growth (from mid-flowering stage) and yield parameters and each trait is expressed as a percentage of the Columbia control. The results strongly support an enhanced growth phenotype. This enhanced growth phenotype is present at all growth stages. At the vegetative stage, all ABA^S transgenic plants showed an increase in leaf number relative to that of the wild type with four of the eight lines showing a statistically significant difference. The two ABA^{Wt} lines showed the same or fewer leaves relative to wild type.

At the bolting stage ABA^S transgenics showed an increase in leaf number but plants were shorter at this stage (first open flower) than controls. The shoot fresh weight of transgenics was significantly increased relative to that of controls, ranging from 80% to 342% of the wild type. The ABA^S transgenics displayed a delay in flowering from one to three days. The ABA^{Wt} transgenics did not show delayed flowering, increased shoot fresh weight or increased height.

At the flowering stage of development the enhanced growth phenotype is maintained (greater leaf number and fresh weight), however, there were no observable differences in plant height indicating that transgenics bolt shorter but reach same final plant height.

Of particular significance is the observation, that under these conditions (biotic stress due to presence of fungi in the soil) yields of the ABA^S transgenics were significantly higher, ranging from 120% to 229% of the wild type control. The ABA^{Wt} lines showed similar or

slightly reduced yields relative to the Columbia control. This finding indicates that ABA^S transgenic lines are affected less by the biotic stress. This observation has been confirmed, where 5 of the drought tolerant lines were grown in contaminated soil to maturity. The seed yields of transgenic lines, even though greatly reduced relative to optimal conditions, were 2.5 to 4.5 fold higher than those of Columbia wild type (Table 25).

Table 25. Seed yield of pBI121-AtCPP lines grown in contaminated soil. Values in bold indicate statistical differences at p=0.05

Line	ABA sensitivity	Seed Yield per plant (g)	% of Columbia
156	ABA^S	**0.33 ± 0.04**	316%
23	ABA^S	**0.35 ± 0.05**	336%
76	ABA^S	**0.31 ± 0.04**	296%
84	ABA^S	**0.25 ± 0.33**	237%
9	ABA^S	**0.48 ± 0.05**	455%
Columbia	ABA^{Wt}	0.11 ± 0.03	

40b) **pBI121-AtCPP early seedling growth:**

Four ABA^S and one ABA^{Wt}line plus Columbia were examined for early seedling growth on agar plates. Twenty seeds were plated in a line on agar plates containing 50% MS with 1% sucrose and vitamins and 6 plates per line were used. Plates were placed on slants, which allowed roots to grow downwards. Root length was measured on 7-day old seedlings and shoot and root biomass determined on 11-day old seedlings. Two of the ABA^S transgenic lines had significantly longer roots and all 4 ABA^S lines had shoot dry weights 114% to 123% of controls and root dry weights of 116% to 151% of controls.As a result, the shoot biomass to rootbiomass ratios were slightly reduced in transgenics. These results indicate that enhanced growth of these transgenics is evident in the early growth stage, shortly after germination, and the root growth is more enhanced relative to shoot growth. In a different experiment seedlings were pulled out of agar and roots were stained with toluidine blue to show their structure. Figure 13 shows that transgenic lines had more extensive lateral root system, which would account for greater root biomass.

40c) **pRD29A-HP-AtCPP optimal growth characteristics**

An optimal growth study has been conducted with 10 lines as described before. Vegetative growth data showed that two of the lines (12 and 9) had significantly more leaves and seven of the lines (12, 22, 23, 47, 82, 9) had significantly greater shoot biomass. Bolting data showed that eight of the lines (12, 22, 23, 47, 82, 9, 90, 93) were significantly delayed in flowering by one to two days, and seven of the lines were significantly shorter than Columbia at first open flower. All of the lines except 42 and 7 had significantly greater number of rosette leaves and shoot FW and this trend is maintained into the mid-flowering harvest (Figure 38). The plant height, however, by mid-flowering harvest was not significantly different between the transgenic lines and control. All the lines that showed this enhanced growth also showed drought tolerance and ABA sensitivity.

Example 41. Ultrastructure pBI121-AtCPP

Two of the drought tolerant and ABA^S lines (35 and 76) plus Wt Columbia were used to examine stem and root cross-sections for any differences in ultrastructure. Free hand sections of mature stems (plants flowering for 10days) were obtained from above the first node, stained with toluidine blue and preserved with glycerol. The stems of transgenic plants appeared to have more dense cellular structure and contain one or two more vascular bundles than those of Columbia Wt indicating more enhanced water and nutrient transport system.

Leaf disks were taken and fresh weights determined. Transgenic leaf disks were significantly heavier, 20-24% greater than corresponding wild type controls. This increase is believed to be as a result of a thicker leaf.

Example 42. Cold stress experiment pBI121-AtCPP

Four drought tolerant, ABA^S lines (156, 23, 35, 76) and one ABA^{Wt}(95) line plus wild type Columbia were included in a cold stress study. Plants were grown in 3" pots one per pot) with 10 replicate pots per line at 22C for 10 days (7 days on agar plates and 4 in soil). The cold stress group was moved into 7°C for 5 days while the optimal group was left at 22C. After 5 days in the cold both cold stress group and the optimal group were harvested for shoot biomass determination. ABA^S and drought tolerant lines had significantly greater shoot biomass than Columbia in both optimal (25 to 39% greater shoot fresh weight) and cold stress groups (18 to 44% greater shoot DW) (Table 26). Results of an eight-day cold stress showed that differences between the transgenic lines and Columbia were even more pronounced (53 to 61% greater

shoot fresh weight). This result indicates greater plant vigor and better ability of transgenics to cope with cold stress.

Table 26. Shoot fresh weight of optimal and cold stressed (5C for 5d) pBI121-AtCPP. Values in bold indicate statistical difference at p=0.05

Line	ABA sensitivity	Optimal shoot FW		Cold stress shoot FW	
		mg	% of Columbia	mg	% of Columbia
156	ABA^S	**95.4 ± 3.7**	137%	**23.1 0.7**	118%
23	ABA^S	**96.3 ± 3.9**	139%	**28.3 1.5**	144%
35	ABA^S	**87.0 ± 1.7**	125%	**25.3 1.4**	130%
76	ABA^S	**94.7 ± 2.2**	136%	**27.3 1.5**	140%
95	ABAWt	67 ± 2.4	96%	21.4 1.0	109%
Columbia	ABAWt	69 ± 1.9		19.6 1.1	

Example 43. Drought stress under high temperature pBI121-AtCPP

A drought stress experiment was conducted as described above except that day temperature of 32°C (16hr) and night temperature of 22°C (8hr) was maintained. These temperatures were achieved daily over a 2hr ramping period. Four ABA^S and one ABA^{Wt} line plus Columbia were included. Plants were monitored daily for water loss and soil water content and after 5 days of drought treatment half of the plants were harvested and the other half was re-watered and allowed to recover for four days. Shoots were harvested and shoot fresh weight determined. The results (Table 27) of this experiment showed that previously identified drought tolerant lines maintained their drought tolerant phenotype at high temperature and were able to recover well from the drought stress at high temperature

Table 27. Soil water content on day 2 and water lost in 2 days/final shoot dry weight plus recovery shoot FW after 5days of drought stress at 32C day and 22C night temperatures. Values in bold indicate significant differences from the Columbia control.

line	ABA sensitivity	soil water content day 2	water lost in 2d/shoot DW	recovered shoot FW (g)
136	ABA^S	50.4 ± 1.1	**485.7** ± 18.5	**1.30** ± 0.04
146	ABA^S	**52.1** ± 1.0	**504.5** ± 7.9	**1.15** ± 0.04
35	ABA^S	**52.2** ± 0.8	**502.8** ±15.8	**1.19** ± 0.02
76	ABA^S	**52.1** ± 0.6	**435.6** ± 10.5	**1.11** ± 0.03
95	ABAWt	50.0 ± 0.9	518.2 ± 13.0	0.86 ± 0.03
Columbia	ABAWt	48.6 ± 0.6	559.7 ± 19.0	0.84 ± 0.03

Example 44. Heat stress and seed yield pBI121-AtCPP

Two ABA^S lines and one ABA^{Wt}line plus Columbia were examined for the effect of heat stress during flowering on the final seed yield. Plants were grown in 4 inch pots (one/pot) as described above and 9 days from first open flower the temperature was ramped from 22 C to 43C over 2 hours and plants were kept at 43C for 2hr. Temperature was then ramped back to 22C over 2 hours and plants were grown under optimal conditions until maturity. The seed yields from this experiment are shown in Table 28. One of the drought tolerant lines (35) had significantly greater yield than Columbia.

Table 28. Seed yield of pBI121-AtCPP lines after two hour 43C heat stress 9 days from first open flower. Values in bold are statistically significant from Columbia.

Line	ABA sensitivity	seed yield (g/plant)	seed yield (% of col.)
35	ABA^S	**0.55** ± 0.05	347%
76	ABA^S	0.24 ± 0.03	148%
95	ABAWt	0.11 ± 0.02	69%
Columbia	ABAWt	0.16 ± 0.03	

The effect of heat shock on lines of pBI121-AtCPP at the early flowering stage was assessed. Three ABA^S lines (76, 136, 97) a ABA^{Wt}line (95) and a Columbia wild type control

were seeded in 128 cell flats, one flat per line. At the early flowering stage flats were exposed to a temperature of 46.8°C for 50 minutes and then returned to normal growth conditions. Lack of continued growth from main meristems was defined as main meristem death and scored for each line. Data is shown in Table 29.

Table 29. Meristem death due to heat shock

Line	Wt	95	76	136	97
% Death	91	97	79	59	18

Example 45. Stomata density determinations pBI121AtCPP

Two ABA^S lines (76 and 35) plus Columbia were examined for stomata density on the upper and lower leaf surface. Nail polish imprints of the upper and lower epidermis were obtained from a fully expanded leaf #5. These imprints were analyzed under the microscope and the number of stomata per 8.7×10^{-8} m^2 were counted. There were no significant differences found between transgenics and Columbia in the stomata of the upper or lower epidermis (Table 30). The increases seen in drought tolerance and reduced water loss is not attributable to a reduced number of leaf stomata.

Table 30. Stomata numbers per 8.7×10^{-8} m^2 of abaxial and adaxial epidermis of fully expanded leaf #5 in pBI121AtCPP.

Line	ABA sensitivity	stomata on upper epidermis	stomata on lower epidermis
35	ABA^S	68 ± 5	103 ± 7
76	ABA^S	58 ± 6	120 ± 16
Columbia	ABAWt	57 ± 6	116 ± 11

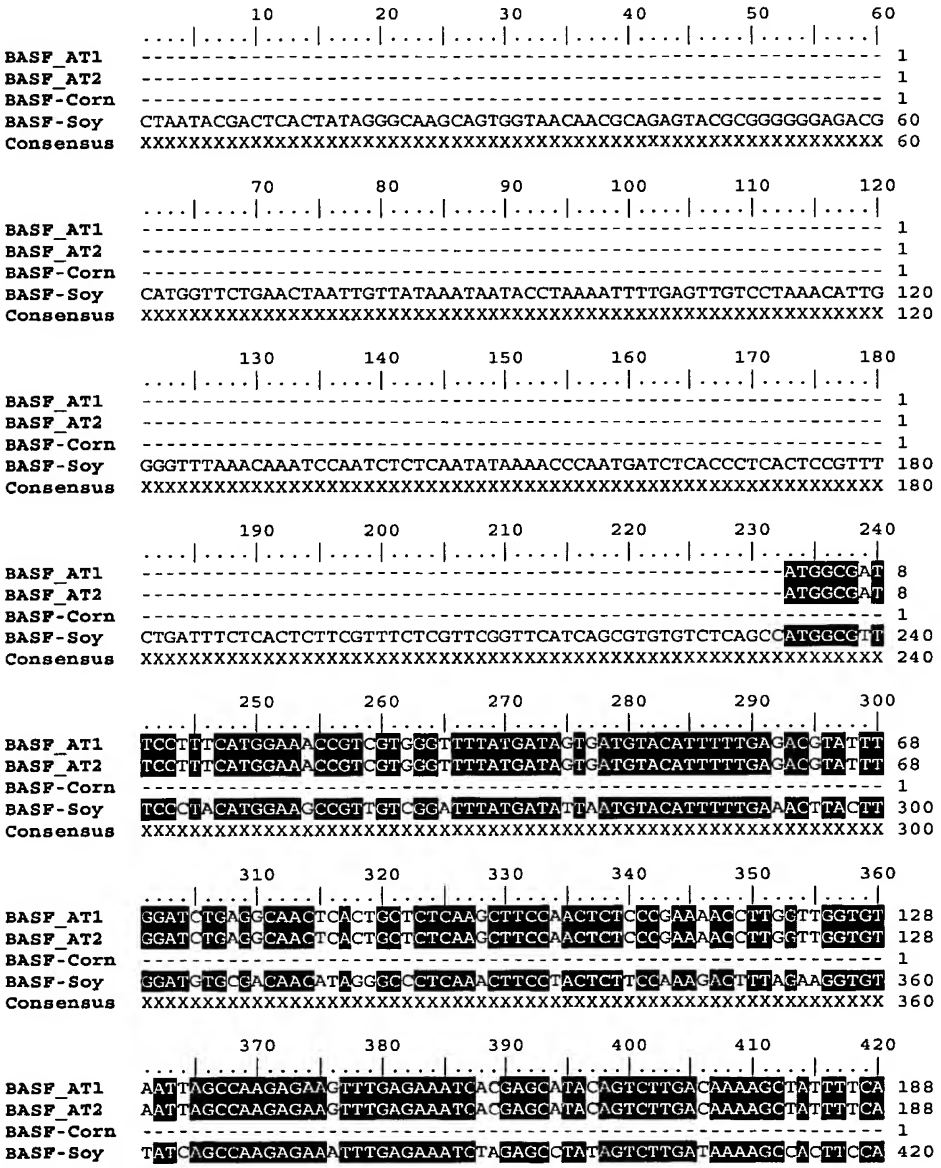
Example 46. CPP Consensus Sequences

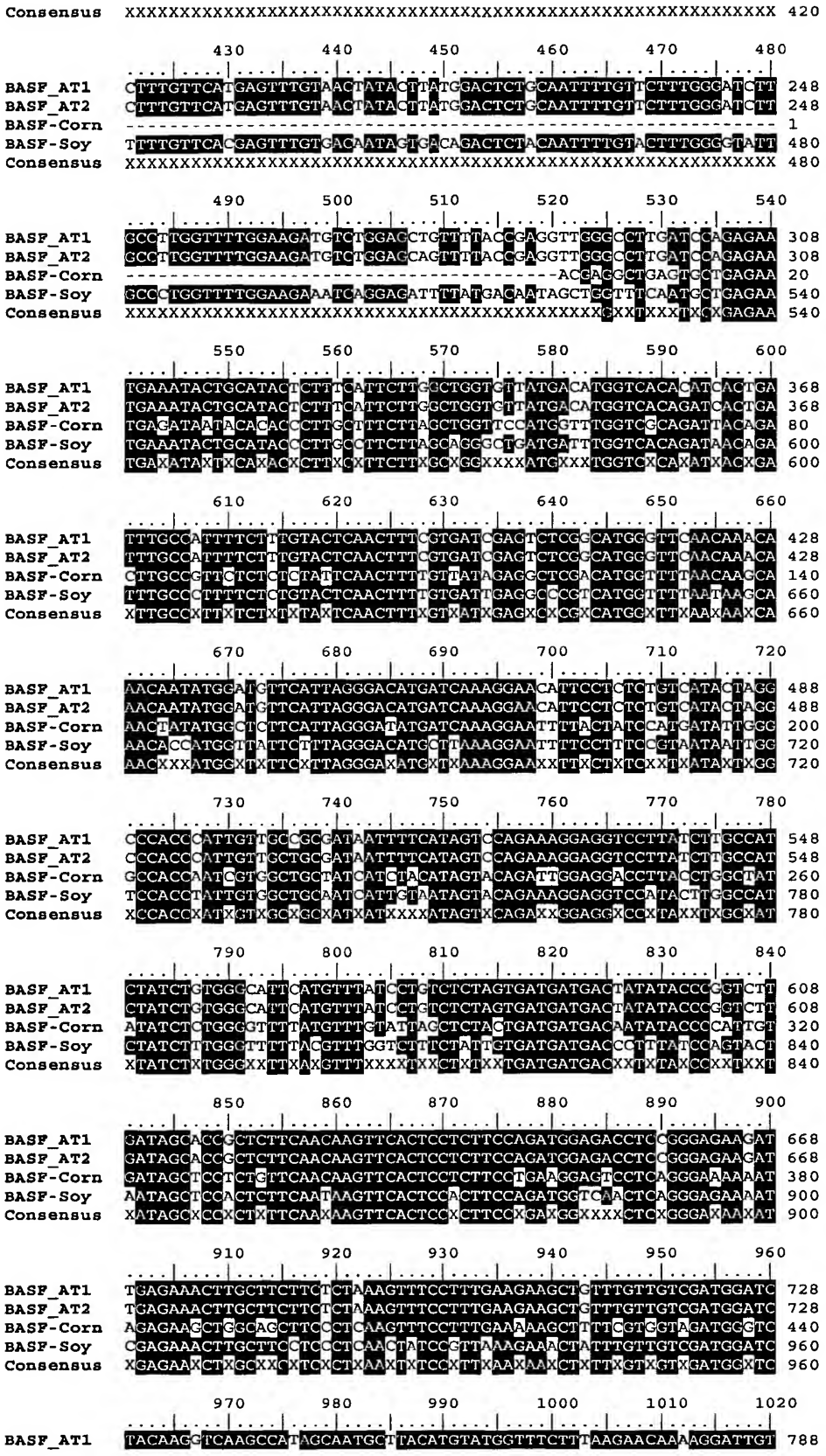
Also included in the invention is the CPP consensus sequences. The consensus sequences were generated by alignment of the CPP polypeptide and nucleic acid ssequences as well as sequences homogous using the program BioEdit.

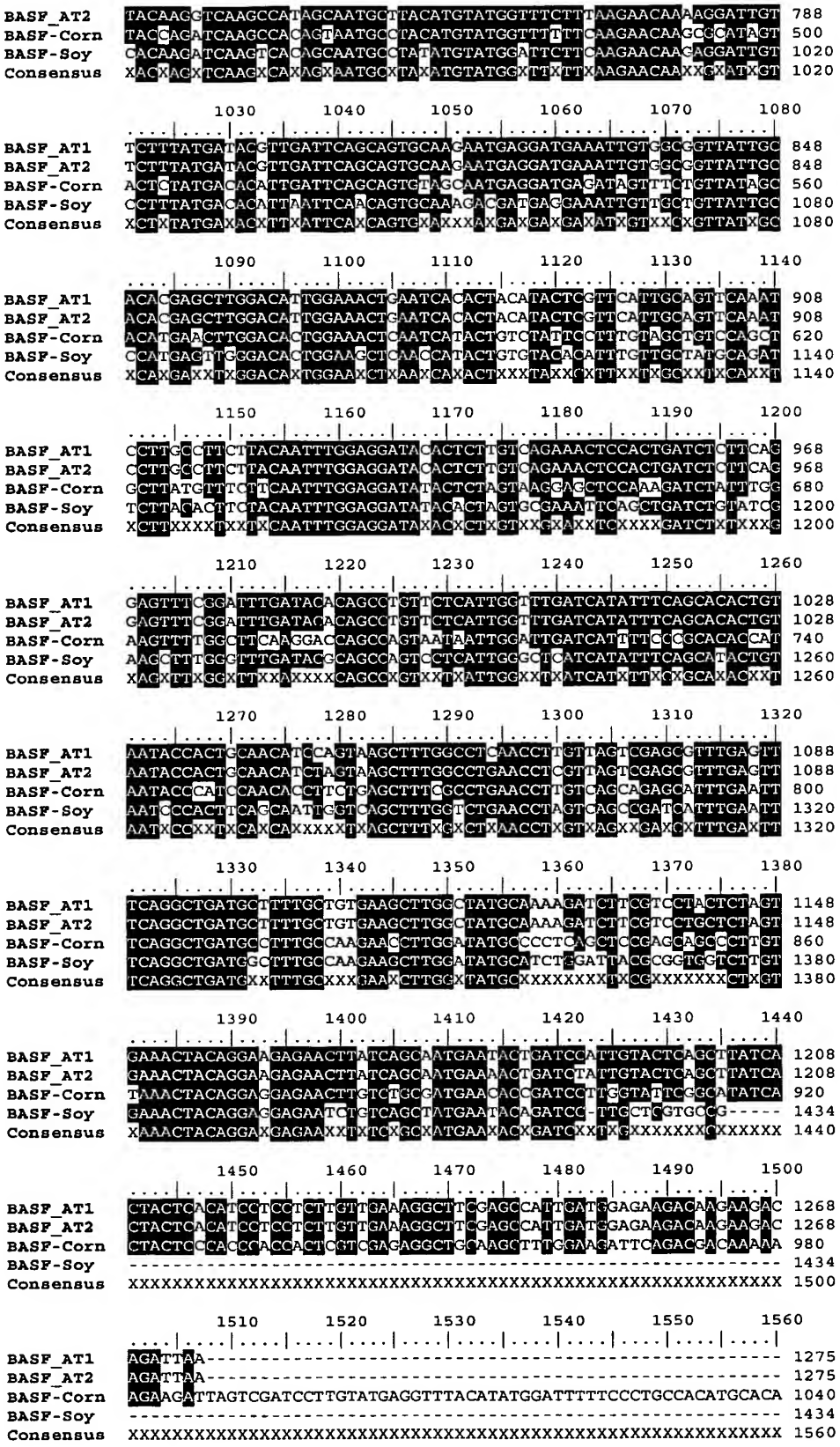
The "x" in the consensus sequence represents any amino acid or nucleotide. Preferably "x" a conservative amino acid or nucleotide substitution. More preferably, "x" is the most amino acid or nucleotide most prevalent at a given postion. For example, the amino acid at postion 145 of SEQ ID NO: 168 is a proline as it occurs 66% of the time.

Table 31. ClustalW Analysis of BASF Nucleic Acids

1) BASF_AT1 (SEQ ID NO:116)
2) BASF_AT2 (SEQ ID NO:118)
3) BASF-Corn (SEQ ID NO:120)
4) BASF-Soy (SEQ ID NO:122)
5) Consensus (SEQ ID NO:163)



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BASF-Corn TACCAGATCAAGCCACAGTAATGCCTACATGTATGGTTTTTTCAAGAACAAGCGCATAGT 500
BASF-Soy CACAAGATCAAGTCACAGCAATGCCTATATGTATGGATTCTTCAAGAACAAGAGGATTGT 1020
Consensus XACXACXTCAAGXCAXAGXAATGCXTAXATGTATGGXTTXTTXAAGAACAAXXGXATXGT 1020
1030 1040 1050 1060 1070 1080
BASF_AT1 TCTTTATGATACGTTGATTCAGCAGTGCAACAATGAGGATGAAATTGTGGCGGTTATTGC 848
BASF_AT2 TCTTTATGATACGTTGATTCAGCAGTGCAAGAATGAGGATGAAATTGTGGCGGTTATTGC 848
BASF-Corn ACTCTATGACACATTGATTCAGCAGTGTAGCAATGAGGATGAGATAGTTTCTGTTATAGC 560
BASF-Soy CCTTTATGACACATTAATTCAACAGTGCAAAGACGATGACGAAATTGTTGCTGTTATTGC 1080
Consensus XCTXTATGAXACXTTXATTCAXCAGTGXAXXXAXGAXGAXGAXATXGTXXCXGTTATXGC 1080
1090 1100 1110 1120 1130 1140
BASF_AT1 ACACGAGCTTGGACATTGGAAACTGAATCACACTACATACTCGTTCATTGCAGTTCAAAT 908
BASF_AT2 ACACGAGCTTGGACATTGGAAACTGAATCACACTACATACTCGTTCATTGCAGTTCAAAT 908
BASF-Corn ACATGAACTTGGACACTGGAAACTCAATCATACTGTCTATTCCTTTGTAGCTGTCCAGGT 620
BASF-Soy CCATGAGTTGGGACACTGGAAGCTCAACCATACTGTGTAGACATTTGTTGCTATGCACAT 1140
Consensus XCAXGAXXTXGGACAXTGGAAXCTXAAXCAXACTXXXTAXXCXTTXXTXCCXXTXCAXXT 1140
1150 1160 1170 1180 1190 1200
BASF_AT1 CCTTGCCTTCTTACAATTTGGAGGATACACTCTTGTCAGAAACTCCACTGATCTCTTCAG 968
BASF_AT2 CCTTGCCTTCTTACAATTTGGAGGATACACTCTTGTCAGAAACTCCACTGATCTCTTCAG 968
BASF-Corn GCTTATGTTTCTTCAATTTGGAGGATATACTCTAGTAAGCAGCTCCAAAGATCTATTTGG 680
BASF-Soy TCTTAGACTTCTACAATTTGGAGGATATACACTAGTGCGAAATTCAGCTGATCTGTATCG 1200
Consensus XCTTXXXXTXXTXCAATTTGGAGGATAXACXCTXGTXXGXAXXTCXXXXGATCTXTXXXG 1200
1210 1220 1230 1240 1250 1260
BASF_AT1 GAGTTTCGGATTTGATACACAGCCTGTTCTCATTGGTTTGATCATATTTCAGCACACTGT 1028
BASF_AT2 GAGTTTCGGATTTGATACACAGCCTGTTCTCATTGGTTTGATCATATTTCAGCACACTGT 1028
BASF-Corn AAGTTTTGGCTTCAAGGACCAGCCAGTAATAATTGGATTGATCATTTTCCCGCACACCAT 740
BASF-Soy AAGCTTTGGGTTTGATACGCAGCCAGTCCTCATTGGGCTCATCATATTTCAGCATACTGT 1260
Consensus XAGXTTXGGXTTXXAXXXXCAGCCXGTXXTXATTGGXXTXATCATXTTXGXGCAXACXXT 1260
1270 1280 1290 1300 1310 1320
BASF_AT1 AATACCACTGCAACATCCAGTAAGCTTTGGCCTCAACCTTGTTAGTCGAGCGTTTGAGTT 1088
BASF_AT2 AATACCACTGCAACATCTAGTAAGCTTTGGCCTGAACCTCGTTAGTCGAGCGTTTGAGTT 1088
BASF-Corn AATACCCATCCAACACCTTCTGAGCTTTCGCCTGAACCTTGTCAGCAGAGCATTTGAATT 800
BASF-Soy AATCCCACTTCAGCAATTGGTCAGCTTTGGTCTGAACCTAGTCAGCCGATCATTTGAATT 1320
Consensus AATXCCXXTXCAXCAXXXXXTXAGCTTTXGXCTXAACCTXGTXAGXXGAXGXTTTGAXTT 1320
1330 1340 1350 1360 1370 1380
BASF_AT1 TCAGGCTGATGCTTTTGCTGTGAAGCTTGGCTATGCAAAAGATCTTCGTCCTACTCTAGT 1148
BASF_AT2 TCAGGCTGATGCTTTTGCTGTGAAGCTTGGCTATGCAAAAGATCTTCGTCCTGCTCTAGT 1148
BASF-Corn TCAGGCTGATGCCTTTGCCAAGAACCTTGGATATGCCCCTCAGCTCCGAGCAGCCCTTGT 860
BASF-Soy TCAGGCTGATGGCTTTGCCAAGAAGCTTGGATATGCATCTGGATTACGCGGTGGTCTTGT 1380
Consensus TCAGGCTGATGXXTTTGCXXXGAAXCTTGGXTATGCXXXXXXXXTXCGXXXXXXXCTXGT 1380
1390 1400 1410 1420 1430 1440
BASF_AT1 GAAACTACAGGAAGAGAACTTATCAGCAATGAATACTGATCCATTGTACTCAGCTTATCA 1208
BASF_AT2 GAAACTACAGGAAGAGAACTTATCAGCAATGAAAACTGATCTATTGTACTCAGCTTATCA 1208
BASF-Corn TAAACTACAGGAGGAGAACTTGTCTGCGATGAACACCGATCCTTGGTATTCGGCATATCA 920
BASF-Soy GAAACTACAGGAGGAGAATCTGTCAGCTATGAATACAGATCG-TTGCTCGTGCCG----- 1434
Consensus XAAACTACAGGAXGAGAAXXTXTCXGCXATGAAXACXGATCXXTXGXXXXXXXCXXXXXX 1440
1450 1460 1470 1480 1490 1500
BASF_AT1 CTACTCACATCCTCCTCTTGTTGAAAGGCTTCGAGCCATTGATGGAGAAGACAAGAAGAC 1268
BASF_AT2 CTACTCACATCCTCCTCTTGTTGAAAGGCTTCGAGCCATTGATGGAGAAGACAAGAAGAC 1268
BASF-Corn CTACTCCCACCCACCACTCGTCGAGAGGCTGCAAGCTTTGGAAGATTCAGACGACAAAAA 980
BASF-Soy ---------- 1434
Consensus XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 1500
1510 1520 1530 1540 1550 1560
BASF_AT1 AGATTAA---------- 1275
BASF_AT2 AGATTAA---------- 1275
BASF-Corn AGAAGATTAGTCGATCCTTGTATGAGGTTTACATATGGATTTTTCCCTGCCACATGCACA 1040
BASF-Soy ---------- 1434
Consensus XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 1560

```
                1570      1580      1590      1600      1610      1620
               ....|....|....|....|....|....|....|....|....|....|....|
BASF_AT1       ---------------------------------------------------------- 1275
BASF_AT2       ---------------------------------------------------------- 1275
BASF-Corn      CCGATTCAGTGCTTGGATGGTGAGGGTTTTGACATAGGAGTGTTGTCAAAGCTTTAGAGT 1100
BASF-Soy       ---------------------------------------------------------- 1434
Consensus      XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 1620

                1630      1640      1650      1660      1670      1680
               ....|....|....|....|....|....|....|....|....|....|....|
BASF_AT1       ---------------------------------------------------------- 1275
BASF_AT2       ---------------------------------------------------------- 1275
BASF-Corn      GCATCTTTCGGTCAGGTGCAACAGCCTTTCGGTCATTGAGACATATAAGCGAATTAGCTA 1160
BASF-Soy       ---------------------------------------------------------- 1434
Consensus      XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 1680

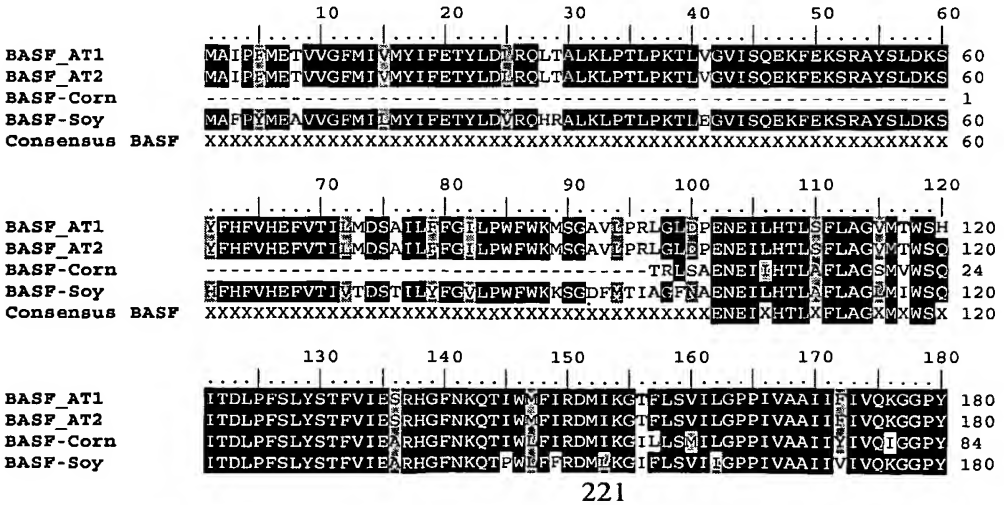
                1690      1700      1710      1720      1730      1740
               ....|....|....|....|....|....|....|....|....|....|....|
BASF_AT1       ---------------------------------------------------------- 1275
BASF_AT2       ---------------------------------------------------------- 1275
BASF-Corn      TTAAAAAAAACAGAACTGTTGCATCAAAAAAAAAAAAAAAAAAGAAACAAAAAAAAAAAA 1220
BASF-Soy       ---------------------------------------------------------- 1434
Consensus      XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 1740

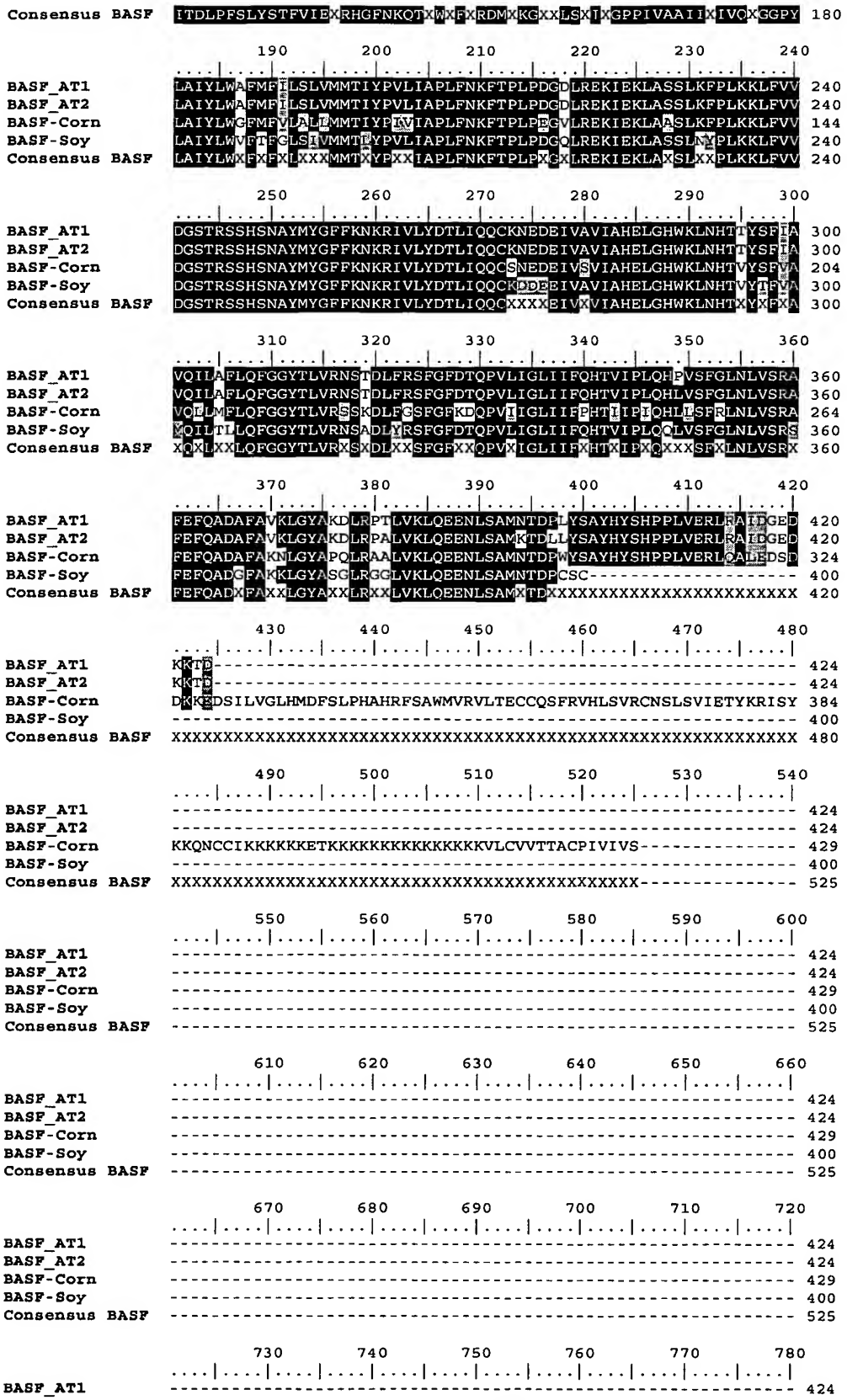
                1750      1760      1770      1780      1790      1800
               ....|....|....|....|....|....|....|....|....|....|....|
BASF_AT1       ---------------------------------------------------------- 1275
BASF_AT2       ---------------------------------------------------------- 1275
BASF-Corn      AAAAAAAAAAAAGAAAAAAAAAAAAAAAAAAAAAAGTGCTCTGCGTTGTTACCACTGCTTG 1280
BASF-Soy       ---------------------------------------------------------- 1434
Consensus      XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 1800

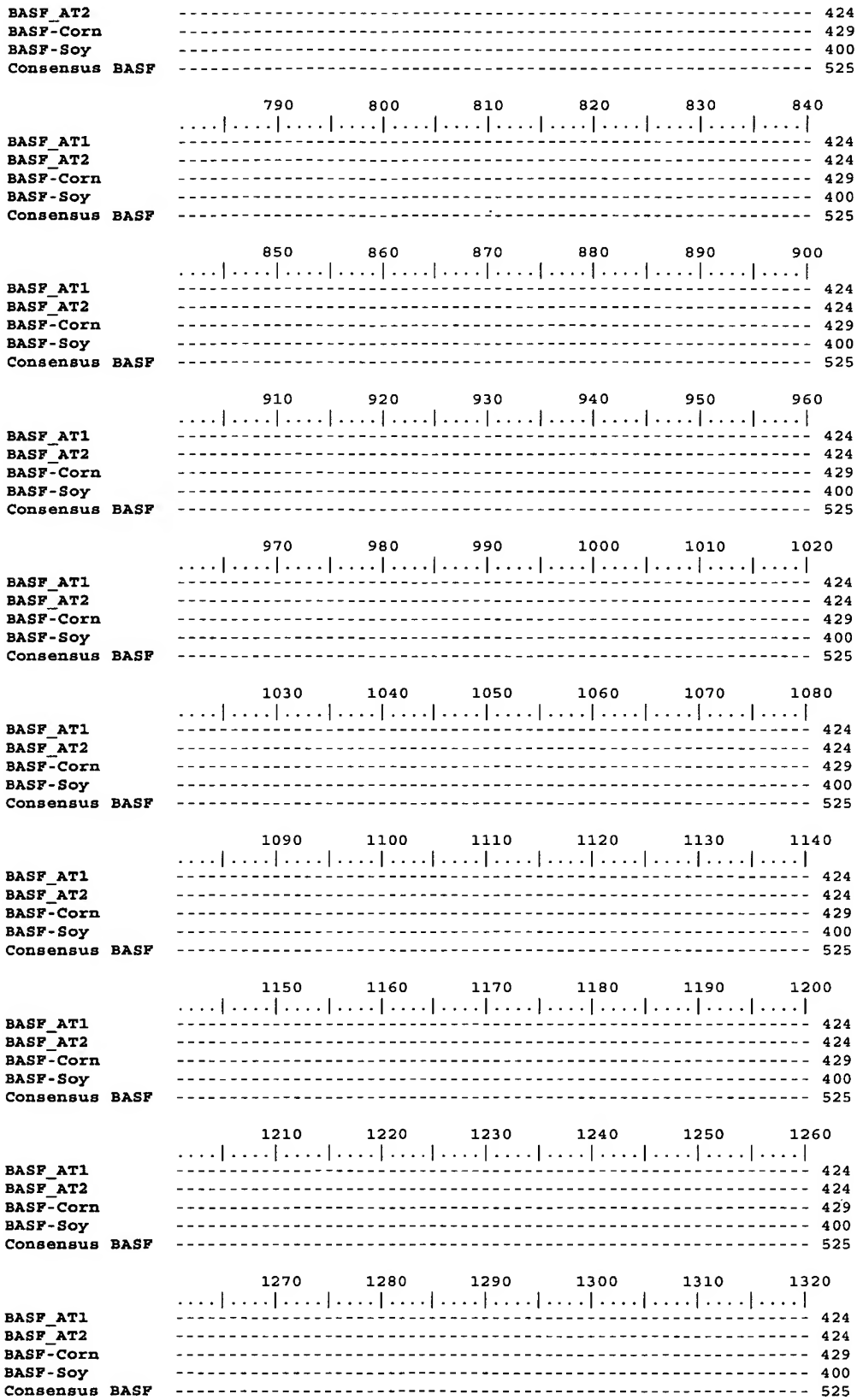
                1810      1820
               ....|....|....|....|.
BASF_AT1       -------------------- 1275
BASF_AT2       -------------------- 1275
BASF-Corn      CCCTATAGTGATCGTATCAGA 1301
BASF-Soy       -------------------- 1434
Consensus      XXXXXXXXXXXXXXXXXXXX 1821
```

Table 32. ClustalW Analysis of BASF Amino Acids

1) BASF_AT1 (SEQ ID NO:117)
2) BASF_AT2 (SEQ ID NO:119)
3) BASF-Corn (SEQ ID NO:121)
4) BASF-Soy (SEQ ID NO:123)
5) Consensus (SEQ ID NO:164)




Consensus BASF
BASF_AT1
BASF_AT2
BASF-Corn
BASF-Soy
Consensus BASF


BASF_AT2 424
BASF-Corn 429
BASF-Soy 400
Consensus BASF 525
790 800 810 820 830 840
BASF_AT1 424
BASF_AT2 424
BASF-Corn 429
BASF-Soy 400
Consensus BASF 525
850 860 870 880 890 900
BASF_AT1 424
BASF_AT2 424
BASF-Corn 429
BASF-Soy 400
Consensus BASF 525
910 920 930 940 950 960
BASF_AT1 424
BASF_AT2 424
BASF-Corn 429
BASF-Soy 400
Consensus BASF 525
970 980 990 1000 1010 1020
BASF_AT1 424
BASF_AT2 424
BASF-Corn 429
BASF-Soy 400
Consensus BASF 525
1030 1040 1050 1060 1070 1080
BASF_AT1 424
BASF_AT2 424
BASF-Corn 429
BASF-Soy 400
Consensus BASF 525
1090 1100 1110 1120 1130 1140
BASF_AT1 424
BASF_AT2 424
BASF-Corn 429
BASF-Soy 400
Consensus BASF 525
1150 1160 1170 1180 1190 1200
BASF_AT1 424
BASF_AT2 424
BASF-Corn 429
BASF-Soy 400
Consensus BASF 525
1210 1220 1230 1240 1250 1260
BASF_AT1 424
BASF_AT2 424
BASF-Corn 429
BASF-Soy 400
Consensus BASF 525
1270 1280 1290 1300 1310 1320
BASF_AT1 424
BASF_AT2 424
BASF-Corn 429
BASF-Soy 400
Consensus BASF 525

```
                    1330      1340      1350      1360      1370      1380
                    ....|....|....|....|....|....|....|....|....|....|....|
BASF_AT1            ------------------------------------------------------------ 424
BASF_AT2            ------------------------------------------------------------ 424
BASF-Corn           ------------------------------------------------------------ 429
BASF-Soy            ------------------------------------------------------------ 400
Consensus BASF      ------------------------------------------------------------ 525
```

Table 33. ClustalW Analysis of Generic Nucleic Acids

1) afc1 (SEQ ID NO:124)
2) AT4g01320 (SEQ ID NO:126)
3) AF007269 (SEQ ID NO:128)
4) Consensus (SEQ ID NO:165)

```
                    10        20        30        40        50        60
                    ....|....|....|....|....|....|....|....|....|....|....|
afc1                ------------------------------------------------------------ 1
AT4g01320           ------------------------------------------------------------ 1
AF007269            ATGGCGATTCCTTTCATGGAAACCGTCGTGGGTAAGCTTCAAAACCTTTTTCTGAGACAT 60
Consensus           XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 60

                    70        80        90        100       110       120
                    ....|....|....|....|....|....|....|....|....|....|....|....|
afc1                ------------------------------------------------------------ 1
AT4g01320           ------------------------------------------------------------ 1
AF007269            TTTACTATCCTGTTTCACTCATCGTATTTCGTTTTTGTTTGGGTTTTGCTTTCTGTGTTG 120
Consensus           XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 120

                    130       140       150       160       170       180
                    ....|....|....|....|....|....|....|....|....|....|....|....|
afc1                ------------------------------------------------------------ 1
AT4g01320           ------------------------------------------------------------ 1
AF007269            TGTGTGTTGAGATTCCATGACTCGTTTGTTTCATATACCATCGTCTCTGCTTCTCGTTTC 180
Consensus           XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 180

                    190       200       210       220       230       240
                    ....|....|....|....|....|....|....|....|....|....|....|....|
afc1                ------------------------------------------------------------ 1
AT4g01320           ------------------------------------------------------------ 1
AF007269            TAAATTTTGTTCTTTTCTAATAGTGCGTACCTTGATCTGAGGTTTTATTACTCCTACTAG 240
Consensus           XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 240

                    250       260       270       280       290       300
                    ....|....|....|....|....|....|....|....|....|....|....|....|
afc1                ------------------------------------------------------------ 1
AT4g01320           ------------------------------------------------------------ 1
AF007269            TTTCTTGTCTTACTCGTGCGTTTGATTTGATTTGAGCTTATGTGATTTCATCATCTCTTC 300
Consensus           XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 300

                    310       320       330       340       350       360
                    ....|....|....|....|....|....|....|....|....|....|....|....|
afc1                ------------------------------------------------------------ 1
AT4g01320           ------------------------------------------------------------ 1
AF007269            CTCGGTTTTAGAATGTACGGAGCTTCTCTGTTAACCAAAATCTAGGATTTGGGAAGAAAA 360
Consensus           XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 360

                    370       380       390       400       410       420
                    ....|....|....|....|....|....|....|....|....|....|....|....|
afc1                ------------------------------------------------------------ 1
AT4g01320           ------------------------------------------------------------ 1
AF007269            GTCGGAGTCTTTTTTTTCCTCATTCCCGATTGGAAATTGAGAATCTTGAAATTTTTCTTT 420
Consensus           XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 420

                    430       440       450       460       470       480
                    ....|....|....|....|....|....|....|....|....|....|....|....|
afc1                ------------------------------------------------------------ 1
AT4g01320           ------------------------------------------------------------ 1
AF007269            GTTCAAGTCATACAGCTTGAGGTTTTGGGTTTTCTTGTCAGGGTATTATTATGTTCGTGA 480
Consensus           XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 480
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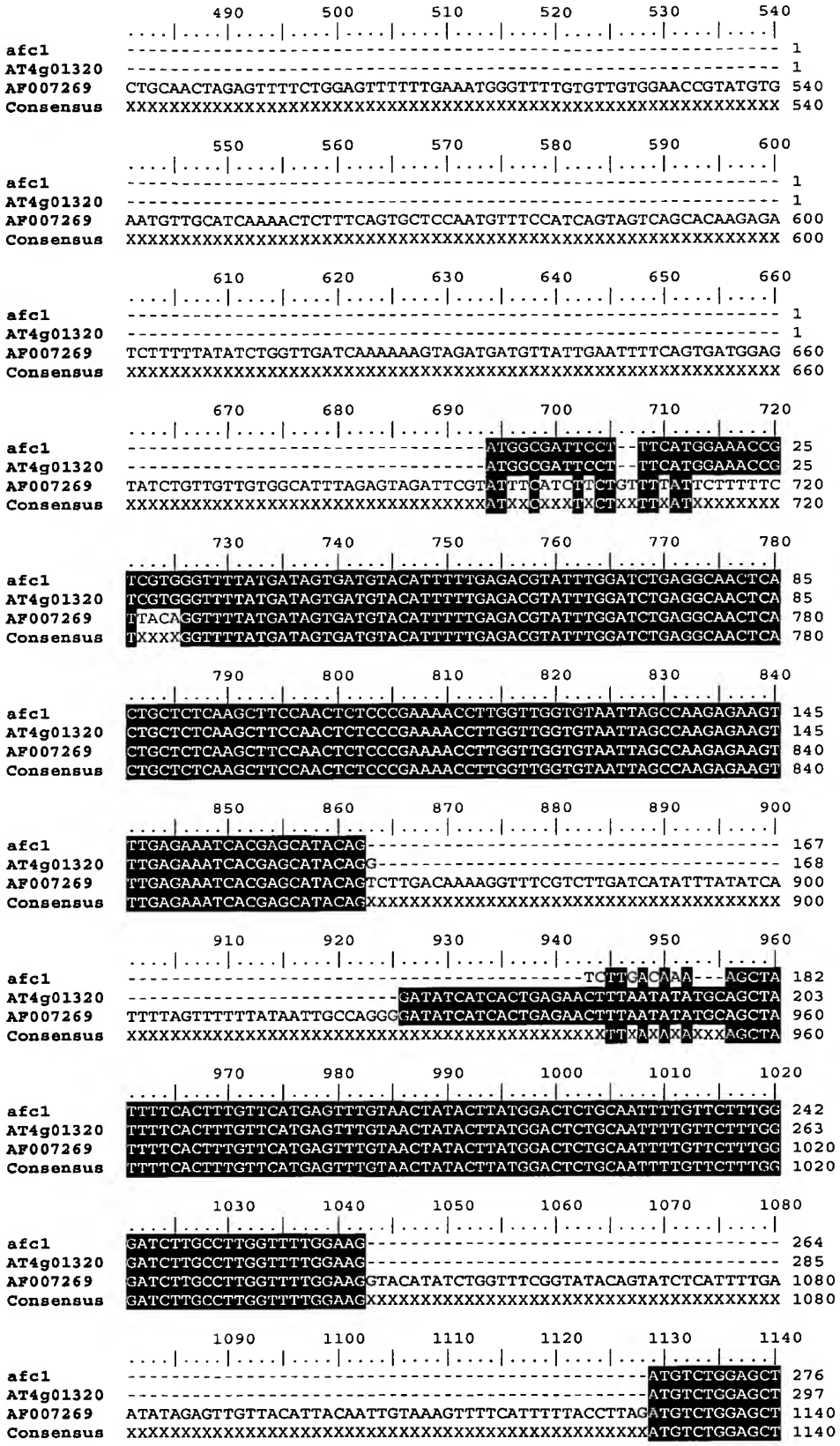
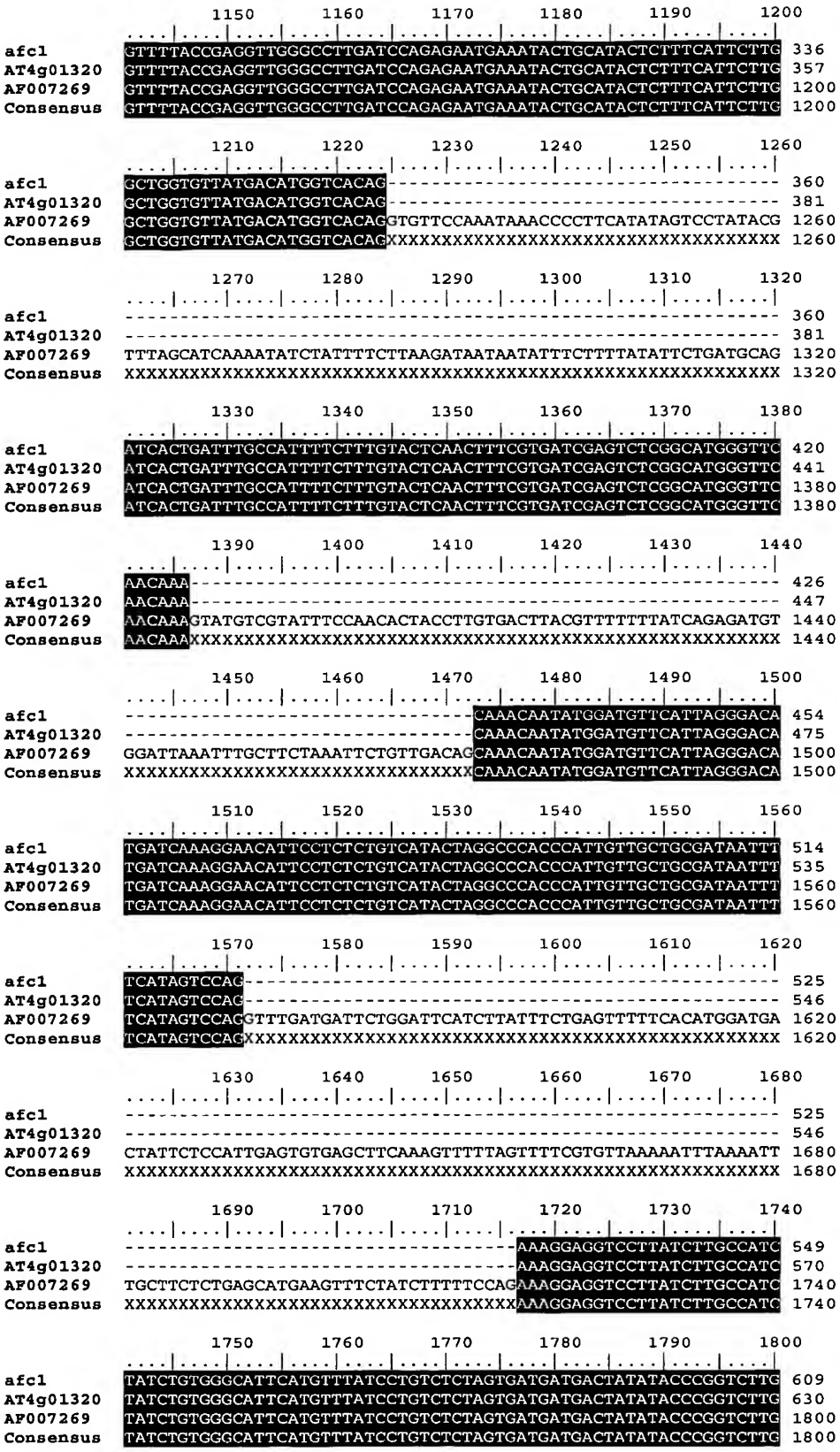

490 500 510 520 530 540
afc1 ---------- 1
AT4g01320 ---------- 1
AF007269 CTGCAACTAGAGTTTTCTGGAGTTTTTTGAAATGGGTTTTGTGTTGTGGAACCGTATGTG 540
Consensus XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 540
550 560 570 580 590 600
afc1 ---------- 1
AT4g01320 ---------- 1
AF007269 AATGTTGCATCAAAACTCTTTCAGTGCTCCAATGTTTCCATCAGTAGTCAGCACAAGAGA 600
Consensus XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 600
610 620 630 640 650 660
afc1 ---------- 1
AT4g01320 ---------- 1
AF007269 TCTTTTTATATCTGGTTGATCAAAAAAGTAGATGATGTTATTGAATTTTCAGTGATGGAG 660
Consensus XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 660
670 680 690 700 710 720
afc1 ATGGCGATTCCT--TTCATGGAAACCG 25
AT4g01320 ATGGCGATTCCT--TTCATGGAAACCG 25
AF007269 TATCTGTTGTTGTGGCATTTAGAGTAGATTCGTATTTCATCTTCTGTTTTATTCTTTTTC 720
Consensus XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXATXXCXXXTXCTXXTTXATXXXXXXXX 720
730 740 750 760 770 780
afc1 TCGTGGGTTTTATGATAGTGATGTACATTTTTGAGACGTATTTGGATCTGAGGCAACTCA 85
AT4g01320 TCGTGGGTTTTATGATAGTGATGTACATTTTTGAGACGTATTTGGATCTGAGGCAACTCA 85
AF007269 TTACAGGTTTTATGATAGTGATGTACATTTTTGAGACGTATTTGGATCTGAGGCAACTCA 780
Consensus TXXXXGGTTTTATGATAGTGATGTACATTTTTGAGACGTATTTGGATCTGAGGCAACTCA 780
790 800 810 820 830 840
afc1 CTGCTCTCAAGCTTCCAACTCTCCCGAAAACCTTGGTTGGTGTAATTAGCCAAGAGAAGT 145
AT4g01320 CTGCTCTCAAGCTTCCAACTCTCCCGAAAACCTTGGTTGGTGTAATTAGCCAAGAGAAGT 145
AF007269 CTGCTCTCAAGCTTCCAACTCTCCCGAAAACCTTGGTTGGTGTAATTAGCCAAGAGAAGT 840
Consensus CTGCTCTCAAGCTTCCAACTCTCCCGAAAACCTTGGTTGGTGTAATTAGCCAAGAGAAGT 840
850 860 870 880 890 900
afc1 TTGAGAAATCACGAGCATACAG---------- 167
AT4g01320 TTGAGAAATCACGAGCATACAGG---------- 168
AF007269 TTGAGAAATCACGAGCATACAGTCTTGACAAAAGGTTTCGTCTTGATCATATTTATATCA 900
Consensus TTGAGAAATCACGAGCATACAGXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 900
910 920 930 940 950 960
afc1 ----------TCTTCACAAA---AGCTA 182
AT4g01320 ----------GATATCATCACTGAGAACTTTAATATATGCAGCTA 203
AF007269 TTTTAGTTTTTTATAATTGCCAGGGGATATCATCACTGAGAACTTTAATATATGCAGCTA 960
Consensus XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXTTXAXAXAXXXAGCTA 960
970 980 990 1000 1010 1020
afc1 TTTTCACTTTGTTCATGAGTTTGTAACTATACTTATGGACTCTGCAATTTTGTTCTTTGG 242
AT4g01320 TTTTCACTTTGTTCATGAGTTTGTAACTATACTTATGGACTCTGCAATTTTGTTCTTTGG 263
AF007269 TTTTCACTTTGTTCATGAGTTTGTAACTATACTTATGGACTCTGCAATTTTGTTCTTTGG 1020
Consensus TTTTCACTTTGTTCATGAGTTTGTAACTATACTTATGGACTCTGCAATTTTGTTCTTTGG 1020
1030 1040 1050 1060 1070 1080
afc1 GATCTTGCCTTGGTTTTGGAAG---------- 264
AT4g01320 GATCTTGCCTTGGTTTTGGAAG---------- 285
AF007269 GATCTTGCCTTGGTTTTGGAAGGTACATATCTGGTTTCGGTATACAGTATCTCATTTTGA 1080
Consensus GATCTTGCCTTGGTTTTGGAAGXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 1080
1090 1100 1110 1120 1130 1140
afc1 ----------ATGTCTGGAGCT 276
AT4g01320 ----------ATGTCTCGAGCT 297
AF007269 ATATAGAGTTGTTACATTACAATTGTAAAGTTTTCATTTTTACCTTAGATGTCTGGAGCT 1140
Consensus XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXATGTCTGGAGCT 1140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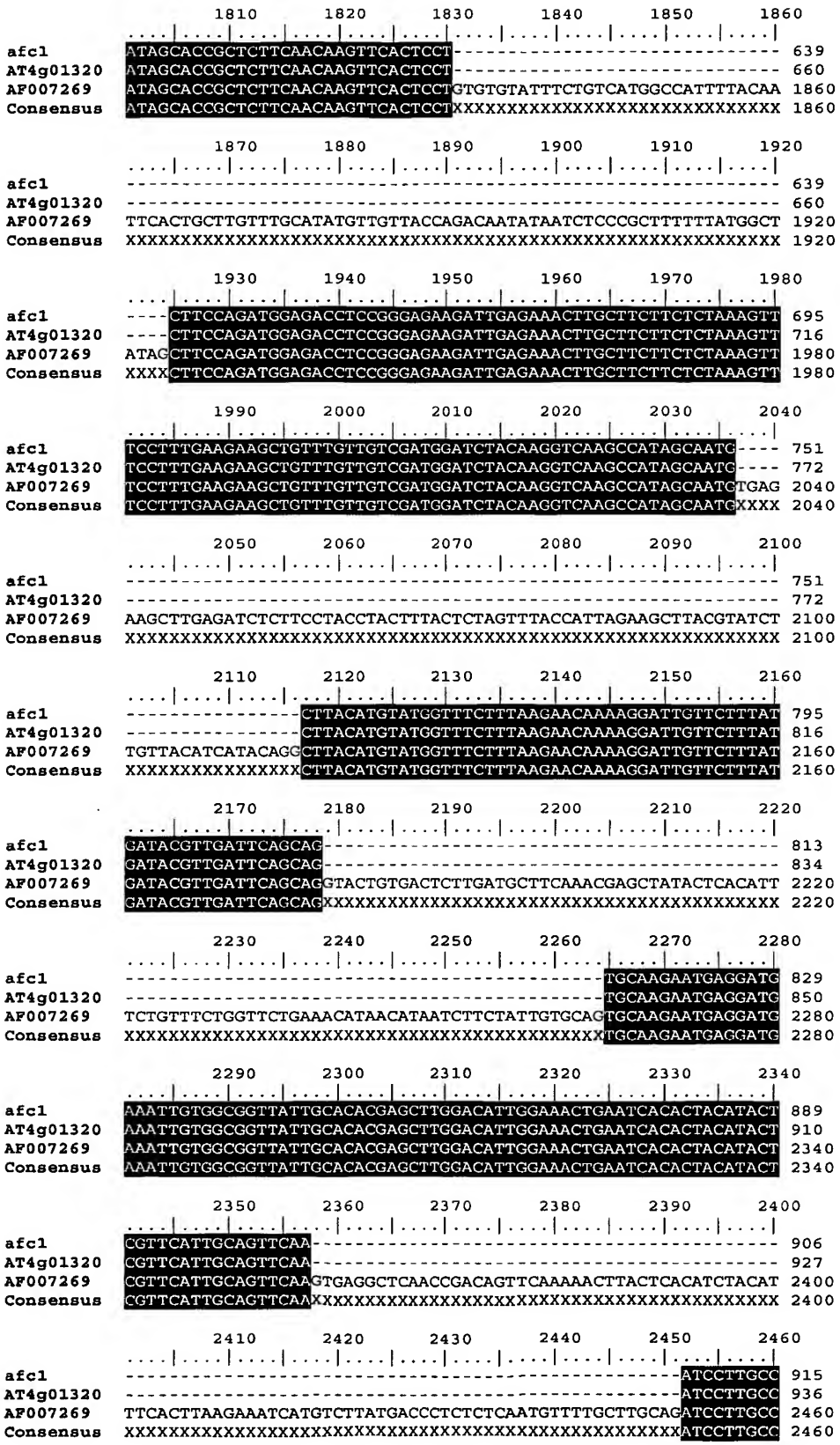
AT4g01320 GTTTTACCGAGGTTGGGCCTTGATCCAGAGAATGAAATACTGCATACTCTTTCATTCTTG 357
AF007269 GTTTTACCGAGGTTGGGCCTTGATCCAGAGAATGAAATACTGCATACTCTTTCATTCTTG 1200
Consensus GTTTTACCGAGGTTGGGCCTTGATCCAGAGAATGAAATACTGCATACTCTTTCATTCTTG 1200
1210 1220 1230 1240 1250 1260
afc1 GCTGGTGTTATGACATGGTCACAG---------- 360
AT4g01320 GCTGGTGTTATGACATGGTCACAG---------- 381
AF007269 GCTGGTGTTATGACATGGTCACAGGTGTTCCAAATAAACCCCTTCATATAGTCCTATACG 1260
Consensus GCTGGTGTTATGACATGGTCACAGXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 1260
1270 1280 1290 1300 1310 1320
afc1 ---------- 360
AT4g01320 ---------- 381
AF007269 TTTAGCATCAAAATATCTATTTTCTTAAGATAATAATATTTCTTTTATATTCTGATGCAG 1320
Consensus XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 1320
1330 1340 1350 1360 1370 1380
afc1 ATCACTGATTTGCCATTTTCTTTGTACTCAACTTTCGTGATCGAGTCTCGGCATGGGTTC 420
AT4g01320 ATCACTGATTTGCCATTTTCTTTGTACTCAACTTTCGTGATCGAGTCTCGGCATGGGTTC 441
AF007269 ATCACTGATTTGCCATTTTCTTTGTACTCAACTTTCGTGATCGAGTCTCGGCATGGGTTC 1380
Consensus ATCACTGATTTGCCATTTTCTTTGTACTCAACTTTCGTGATCGAGTCTCGGCATGGGTTC 1380
1390 1400 1410 1420 1430 1440
afc1 AACAAA---------- 426
AT4g01320 AACAAA---------- 447
AF007269 AACAAAGTATGTCGTATTTCCAACACTACCTTGTGACTTACGTTTTTTTATCAGAGATGT 1440
Consensus AACAAAXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 1440
1450 1460 1470 1480 1490 1500
afc1 ----------CAAACAATATGGATGTTCATTAGGGACA 454
AT4g01320 ----------CAAACAATATGGATGTTCATTAGGGACA 475
AF007269 GGATTAAATTTGCTTCTAAATTCTGTTGACAGCAAACAATATGGATGTTCATTAGGGACA 1500
Consensus XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXCAAACAATATGGATGTTCATTAGGGACA 1500
1510 1520 1530 1540 1550 1560
afc1 TGATCAAAGGAACATTCCTCTCTGTCATACTAGGCCCACCCATTGTTGCTGCGATAATTT 514
AT4g01320 TGATCAAAGGAACATTCCTCTCTGTCATACTAGGCCCACCCATTGTTGCTGCGATAATTT 535
AF007269 TGATCAAAGGAACATTCCTCTCTGTCATACTAGGCCCACCCATTGTTGCTGCGATAATTT 1560
Consensus TGATCAAAGGAACATTCCTCTCTGTCATACTAGGCCCACCCATTGTTGCTGCGATAATTT 1560
1570 1580 1590 1600 1610 1620
afc1 TCATAGTCCAG---------- 525
AT4g01320 TCATAGTCCAG---------- 546
AF007269 TCATAGTCCAGGTTTGATGATTCTGGATTCATCTTATTTCTGAGTTTTTCACATGGATGA 1620
Consensus TCATAGTCCAGXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 1620
1630 1640 1650 1660 1670 1680
afc1 ---------- 525
AT4g01320 ---------- 546
AF007269 CTATTCTCCATTGAGTGTGAGCTTCAAAGTTTTTAGTTTTCGTGTTAAAAATTTAAAATT 1680
Consensus XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 1680
1690 1700 1710 1720 1730 1740
afc1 ----------AAAGGAGGTCCTTATCTTGCCATC 549
AT4g01320 ----------AAAGGAGGTCCTTATCTTGCCATC 570
AF007269 TGCTTCTCTGAGCATGAAGTTTCTATCTTTTTCCAGAAAGGAGGTCCTTATCTTGCCATC 1740
Consensus XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXAAAGGAGGTCCTTATCTTGCCATC 1740
1750 1760 1770 1780 1790 1800
afc1 TATCTGTGGGCATTCATGTTTATCCTGTCTCTAGTGATGATGACTATATACCCGGTCTTG 609
AT4g01320 TATCTGTGGGCATTCATGTTTATCCTGTCTCTAGTGATGATGACTATATACCCGGTCTTG 630
AF007269 TATCTGTGGGCATTCATGTTTATCCTGTCTCTAGTGATGATGACTATATACCCGGTCTTG 1800
Consensus TATCTGTGGGCATTCATGTTTATCCTGTCTCTAGTGATGATGACTATATACCCGGTCTTG 1800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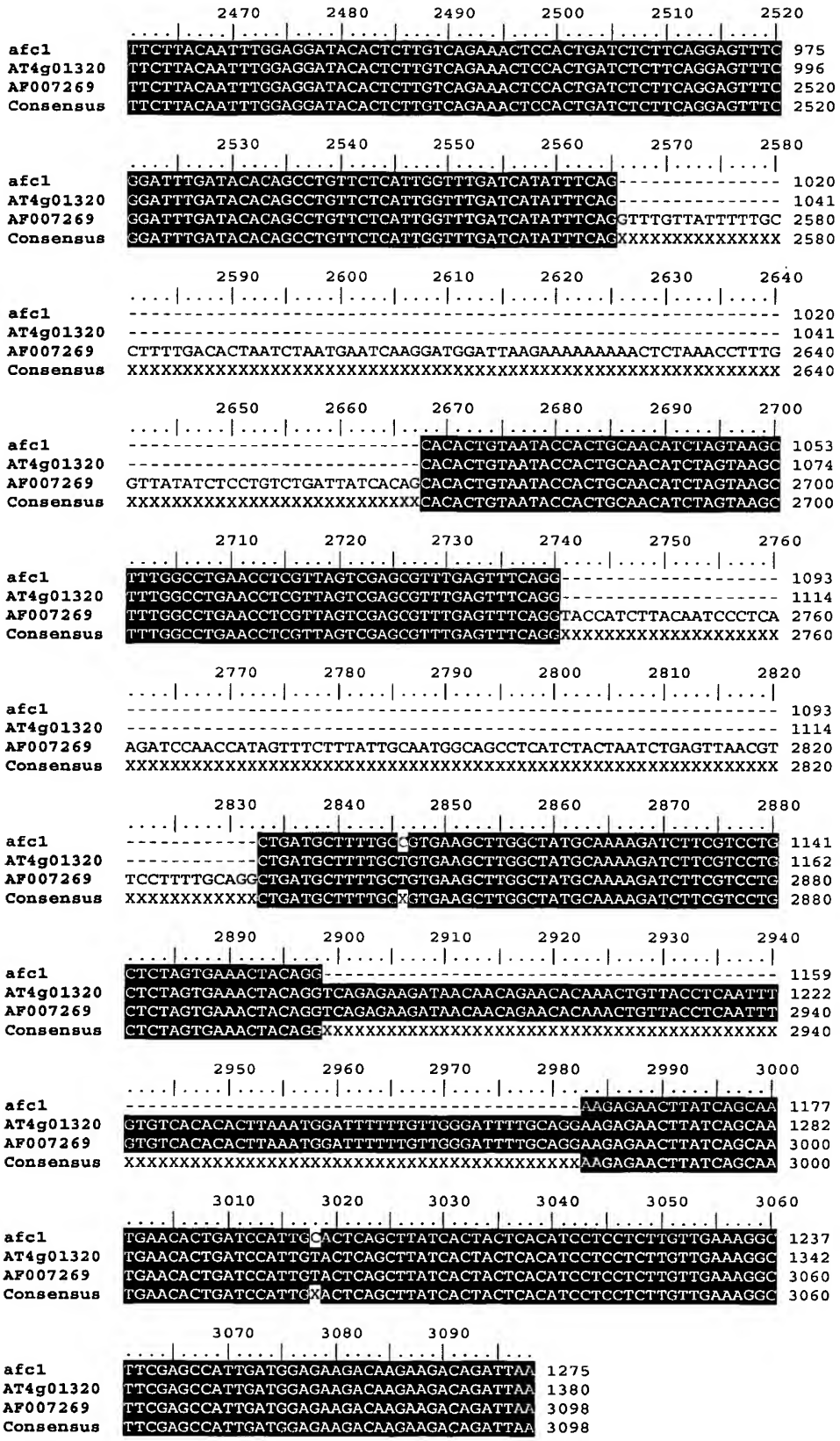
afc1 ATAGCACCGCTCTTCAACAAGTTCACTCCT---------- 639
AT4g01320 ATAGCACCGCTCTTCAACAAGTTCACTCCT---------- 660
AF007269 ATAGCACCGCTCTTCAACAAGTTCACTCCTGTGTGTATTTCTGTCATGGCCATTTTACAA 1860
Consensus ATAGCACCGCTCTTCAACAAGTTCACTCCTXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 1860
1870 1880 1890 1900 1910 1920
afc1 ---------- 639
AT4g01320 ---------- 660
AF007269 TTCACTGCTTGTTTGCATATGTTGTTACCAGACAATATAATCTCCCGCTTTTTTATGGCT 1920
Consensus XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 1920
1930 1940 1950 1960 1970 1980
afc1 ----CTTCCAGATGGAGACCTCCGGGAGAAGATTGAGAAACTTGCTTCTTCTCTAAAGTT 695
AT4g01320 ----CTTCCAGATGGAGACCTCCGGGAGAAGATTGAGAAACTTGCTTCTTCTCTAAAGTT 716
AF007269 ATAGCTTCCAGATGGAGACCTCCGGGAGAAGATTGAGAAACTTGCTTCTTCTCTAAAGTT 1980
Consensus XXXXCTTCCAGATGGAGACCTCCGGGAGAAGATTGAGAAACTTGCTTCTTCTCTAAAGTT 1980
1990 2000 2010 2020 2030 2040
afc1 TCCTTTGAAGAAGCTGTTTGTTGTCGATGGATCTACAAGGTCAAGCCATAGCAATG---- 751
AT4g01320 TCCTTTGAAGAAGCTGTTTGTTGTCGATGGATCTACAAGGTCAAGCCATAGCAATG---- 772
AF007269 TCCTTTGAAGAAGCTGTTTGTTGTCGATGGATCTACAAGGTCAAGCCATAGCAATGTGAG 2040
Consensus TCCTTTGAAGAAGCTGTTTGTTGTCGATGGATCTACAAGGTCAAGCCATAGCAATGXXXX 2040
2050 2060 2070 2080 2090 2100
afc1 ---------- 751
AT4g01320 ---------- 772
AF007269 AAGCTTGAGATCTCTTCCTACCTACTTTACTCTAGTTTACCATTAGAAGCTTACGTATCT 2100
Consensus XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 2100
2110 2120 2130 2140 2150 2160
afc1 ----------CTTACATGTATGGTTTCTTTAAGAACAAAAGGATTGTTCTTTAT 795
AT4g01320 ----------CTTACATGTATGGTTTCTTTAAGAACAAAAGGATTGTTCTTTAT 816
AF007269 TGTTACATCATACAGGCTTACATGTATGGTTTCTTTAAGAACAAAAGGATTGTTCTTTAT 2160
Consensus XXXXXXXXXXXXXXXXCTTACATGTATGGTTTCTTTAAGAACAAAAGGATTGTTCTTTAT 2160
2170 2180 2190 2200 2210 2220
afc1 GATACGTTGATTCAGCAG---------- 813
AT4g01320 GATACGTTGATTCAGCAG---------- 834
AF007269 GATACGTTGATTCAGCAGGTACTGTGACTCTTGATGCTTCAAACGAGCTATACTCACATT 2220
Consensus GATACGTTGATTCAGCAGXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 2220
2230 2240 2250 2260 2270 2280
afc1 ----------TGCAAGAATGAGGATG 829
AT4g01320 ----------TGCAAGAATGAGGATG 850
AF007269 TCTGTTTCTGGTTCTGAAACATAACATAATCTTCTATTGTGCAGTGCAAGAATGAGGATG 2280
Consensus XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXTGCAAGAATGAGGATG 2280
2290 2300 2310 2320 2330 2340
afc1 AAATTGTGGCGGTTATTGCACACGAGCTTGGACATTGGAAACTGAATCACACTACATACT 889
AT4g01320 AAATTGTGGCGGTTATTGCACACGAGCTTGGACATTGGAAACTGAATCACACTACATACT 910
AF007269 AAATTGTGGCGGTTATTGCACACGAGCTTGGACATTGGAAACTGAATCACACTACATACT 2340
Consensus AAATTGTGGCGGTTATTGCACACGAGCTTGGACATTGGAAACTGAATCACACTACATACT 2340
2350 2360 2370 2380 2390 2400
afc1 CGTTCATTGCAGTTCAA---------- 906
AT4g01320 CGTTCATTGCAGTTCAA---------- 927
AF007269 CGTTCATTGCAGTTCAAGTGAGGCTCAACCGACAGTTCAAAAACTTACTCACATCTACAT 2400
Consensus CGTTCATTGCAGTTCAAXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 2400
2410 2420 2430 2440 2450 2460
afc1 ----------ATCCTTGCC 915
AT4g01320 ----------ATCCTTGCC 936
AF007269 TTCACTTAAGAAATCATGTCTTATGACCCTCTCTCAATGTTTTGCTTGCAGATCCTTGCC 2460
Consensus XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXATCCTTGCC 2460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AF007269 TTCTTACAATTTGGAGGATACACTCTTGTCAGAAACTCCACTGATCTCTTCAGGAGTTTC 2520
Consensus TTCTTACAATTTGGAGGATACACTCTTGTCAGAAACTCCACTGATCTCTTCAGGAGTTTC 2520
2530 2540 2550 2560 2570 2580
afc1 GGATTTGATACACAGCCTGTTCTCATTGGTTTGATCATATTTCAG---------- 1020
AT4g01320 GGATTTGATACACAGCCTGTTCTCATTGGTTTGATCATATTTCAG---------- 1041
AF007269 GGATTTGATACACAGCCTGTTCTCATTGGTTTGATCATATTTCAGGTTTGTTATTTTTGC 2580
Consensus GGATTTGATACACAGCCTGTTCTCATTGGTTTGATCATATTTCAGXXXXXXXXXXXXXXX 2580
2590 2600 2610 2620 2630 2640
afc1 ---------- 1020
AT4g01320 ---------- 1041
AF007269 CTTTTGACACTAATCTAATGAATCAAGGATGGATTAAGAAAAAAAAACTCTAAACCTTTG 2640
Consensus XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 2640
2650 2660 2670 2680 2690 2700
afc1 ----------CACACTGTAATACCACTGCAACATCTAGTAAGC 1053
AT4g01320 ----------CACACTGTAATACCACTGCAACATCTAGTAAGC 1074
AF007269 GTTATATCTCCTGTCTGATTATCACAGCACACTGTAATACCACTGCAACATCTAGTAAGC 2700
Consensus XXXXXXXXXXXXXXXXXXXXXXXXXXCACACTGTAATACCACTGCAACATCTAGTAAGC 2700
2710 2720 2730 2740 2750 2760
afc1 TTTGGCCTGAACCTCGTTAGTCGAGCGTTTGAGTTTCAGG---------- 1093
AT4g01320 TTTGGCCTGAACCTCGTTAGTCGAGCGTTTGAGTTTCAGG---------- 1114
AF007269 TTTGGCCTGAACCTCGTTAGTCGAGCGTTTGAGTTTCAGGTACCATCTTACAATCCCTCA 2760
Consensus TTTGGCCTGAACCTCGTTAGTCGAGCGTTTGAGTTTCAGGXXXXXXXXXXXXXXXXXXXX 2760
2770 2780 2790 2800 2810 2820
afc1 ---------- 1093
AT4g01320 ---------- 1114
AF007269 AGATCCAACCATAGTTTCTTTATTGCAATGGCAGCCTCATCTACTAATCTGAGTTAACGT 2820
Consensus XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 2820
2830 2840 2850 2860 2870 2880
afc1 ----------CTGATGCTTTTGCCGTGAAGCTTGGCTATGCAAAAGATCTTCGTCCTG 1141
AT4g01320 ----------CTGATGCTTTTGCTGTGAAGCTTGGCTATGCAAAAGATCTTCGTCCTG 1162
AF007269 TCCTTTTGCAGGCTGATGCTTTTGCTGTGAAGCTTGGCTATGCAAAAGATCTTCGTCCTG 2880
Consensus XXXXXXXXXXXXCTGATGCTTTTGCXGTGAAGCTTGGCTATGCAAAAGATCTTCGTCCTG 2880
2890 2900 2910 2920 2930 2940
afc1 CTCTAGTGAAACTACAGG---------- 1159
AT4g01320 CTCTAGTGAAACTACAGGTCAGAGAAGATAACAACAGAACACAAACTGTTACCTCAATTT 1222
AF007269 CTCTAGTGAAACTACAGGTCAGAGAAGATAACAACAGAACACAAACTGTTACCTCAATTT 2940
Consensus CTCTAGTGAAACTACAGGXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 2940
2950 2960 2970 2980 2990 3000
afc1 ----------AAGAGAACTTATCAGCAA 1177
AT4g01320 GTGTCACACACTTAAATGGATTTTTTGTTGGGATTTTGCAGGAAGAGAACTTATCAGCAA 1282
AF007269 GTGTCACACACTTAAATGGATTTTTTGTTGGGATTTTGCAGGAAGAGAACTTATCAGCAA 3000
Consensus XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXAAGAGAACTTATCAGCAA 3000
3010 3020 3030 3040 3050 3060
afc1 TGAACACTGATCCATTGCACTCAGCTTATCACTACTCACATCCTCCTCTTGTTGAAAGGC 1237
AT4g01320 TGAACACTGATCCATTGTACTCAGCTTATCACTACTCACATCCTCCTCTTGTTGAAAGGC 1342
AF007269 TGAACACTGATCCATTGTACTCAGCTTATCACTACTCACATCCTCCTCTTGTTGAAAGGC 3060
Consensus TGAACACTGATCCATTGXACTCAGCTTATCACTACTCACATCCTCCTCTTGTTGAAAGGC 3060
3070 3080 3090
afc1 TTCGAGCCATTGATGGAGAAGACAAGAAGACAGATTAA 1275
AT4g01320 TTCGAGCCATTGATGGAGAAGACAAGAAGACAGATTAA 1380
AF007269 TTCGAGCCATTGATGGAGAAGACAAGAAGACAGATTAA 3098
Consensus TTCGAGCCATTGATGGAGAAGACAAGAAGACAGATTAA 3098

Table 34. ClustalW Analysis of Generic Amino Acids

1) afc1 (SEQ ID NO:125)
2) AT4g01320 (SEQ ID NO:127)
3) AF007269 (SEQ ID NO:129)
4) Consensus (SEQ ID NO:166)

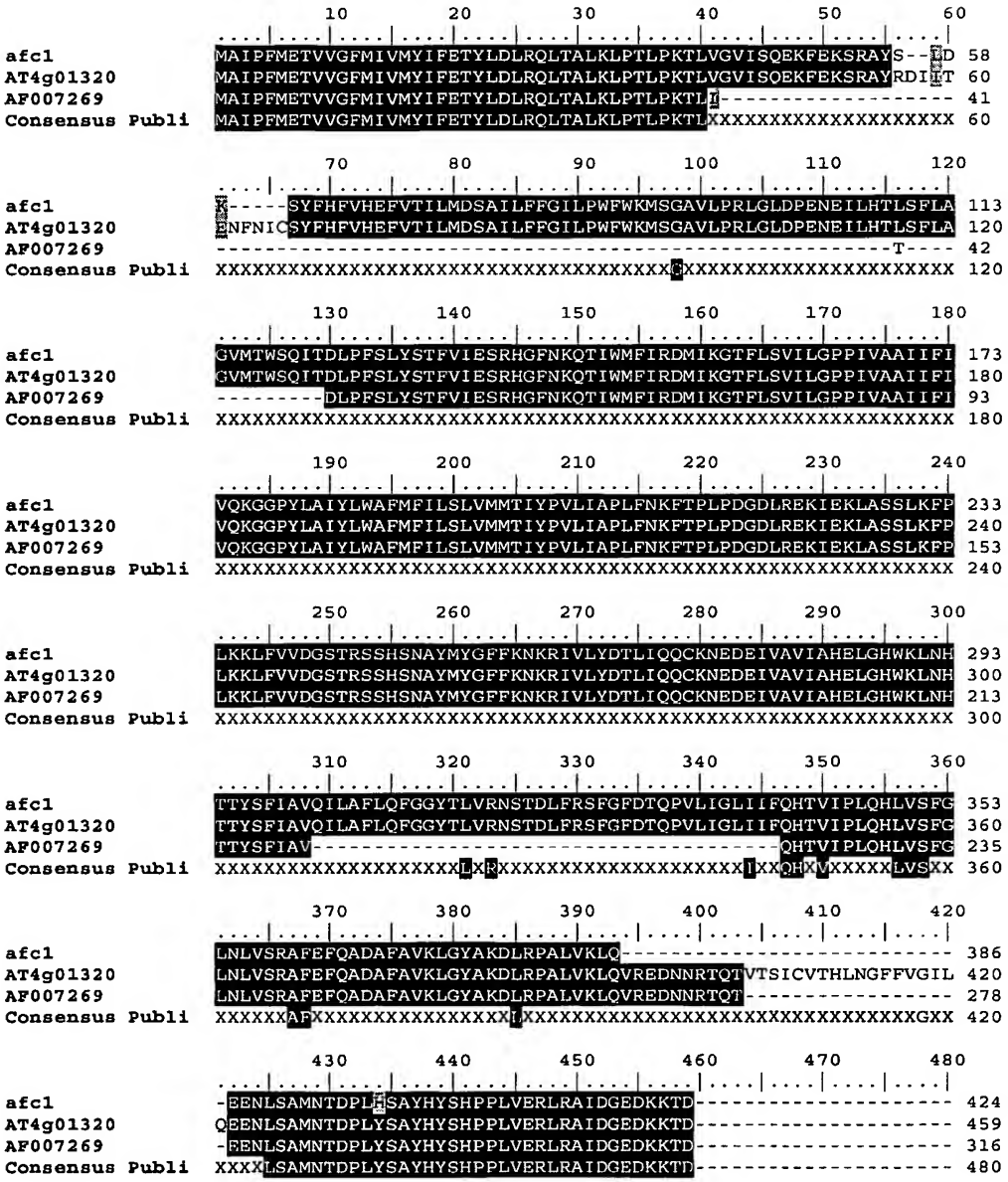
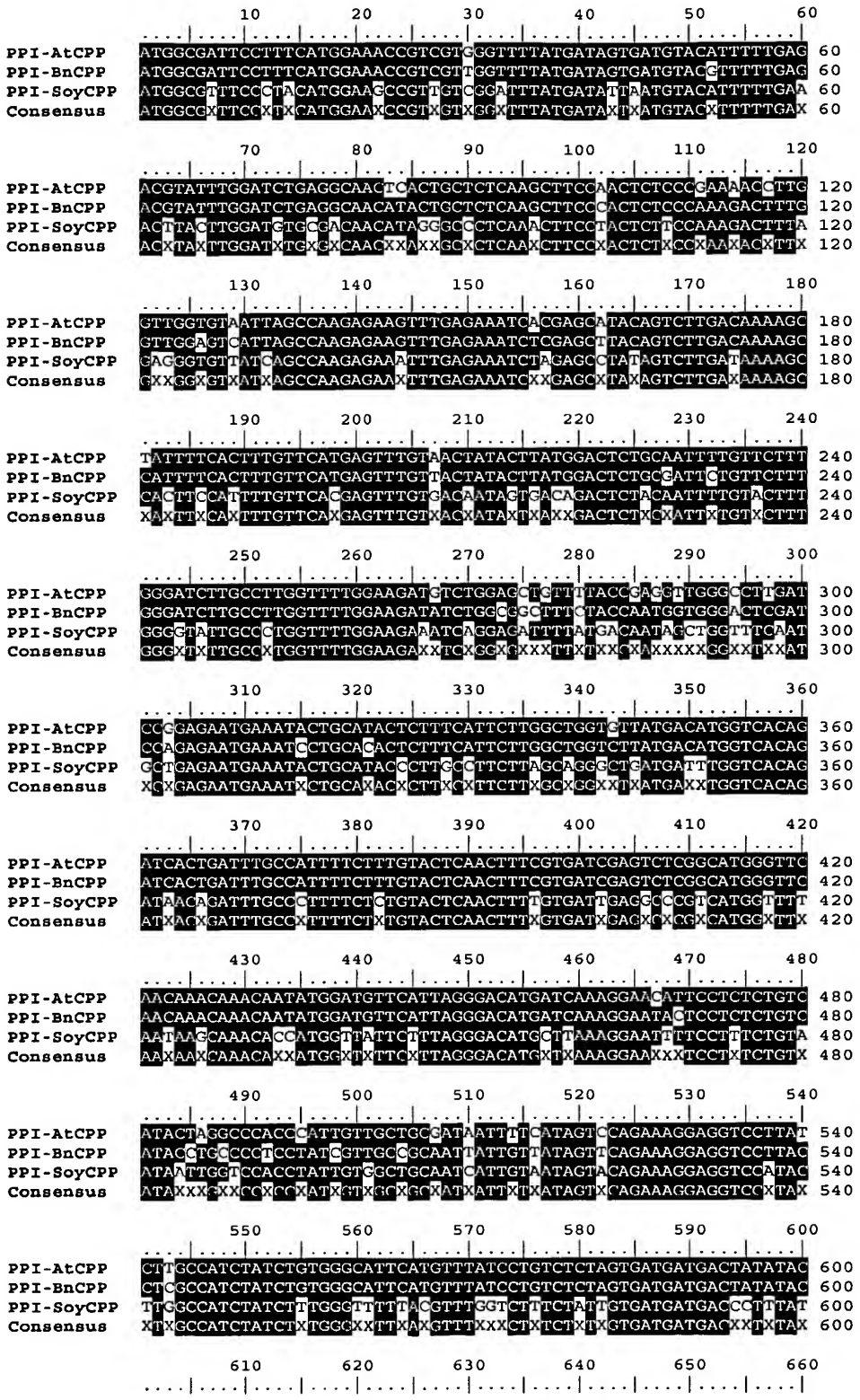
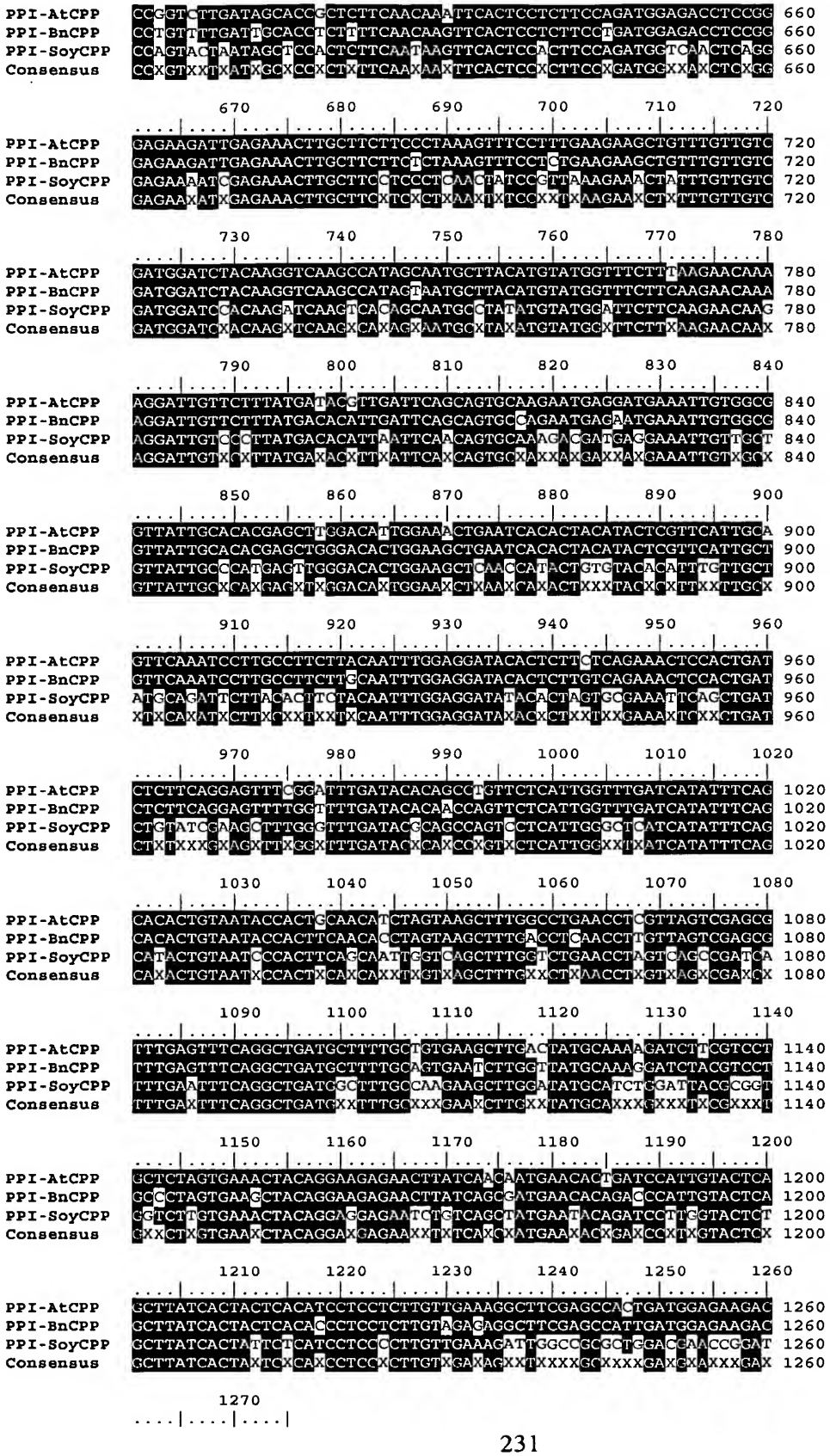


Table 35. ClustalW Analysis of PPI Nucleic Acids

1) PPI-AtCPP (SEQ ID NO:97)

2) PPI-BnCPP (SEQ ID NO:109)
3) PPI-SoyCPP (SEQ ID NO:112)
4) Consensus (SEQ ID NO:167)




PPI-AtCPP
PPI-BnCPP
PPI-SoyCPP
Consensus

```
PPI-AtCPP     AAGAAGACAGATTAA 1275
PPI-BnCPP     AAGAAGACAGATTAA 1275
PPI-SoyCPP    AAGAAGGAAGACTAA 1275
Consensus     AAGAAGXXAGAXTAA 1275
```

Table 36. ClustalW Analysis of PPI Amino Acids

1) PPI-AtCPP (SEQ ID NO:98)
2) PPI-BnCPP (SEQ ID NO:110)
3) PPI-SoyCPP (SEQ ID NO:113)
4) Consensus (SEQ ID NO:168)

```
                        10        20        30        40        50        60
                        |....|....|....|....|....|....|....|....|....|....|....|
PPI-AtCPP               MAFPYMEAVVGFMILMYIFETYLDVRQHRALKLPTLPKTLEGVISQEKFEKSRAYSLDKS 60
PPI-BnCPP               MAIPFMETVVGFMIVMYVFETYLDIRQHTALKLPTLPKTLVGVISQEKFEKSRAYSLDKS 60
PPI-SoyCPP              MAFPYMEAVVGFMILMYIFETYLDVRQHRALKLPTLPKTLEGVISQEKFEKSRAYSLDKS 60
Consensus PPI           MAXPXMEXVVGFMIXMYXFETYLDXRQHXALKLPTLPKTLXGVISQEKFEKSRAYSLDKS 60

                        70        80        90        100       110       120
                        |....|....|....|....|....|....|....|....|....|....|....|
PPI-AtCPP               HFHFVHEFVTIVTDSTILYFGVLPWFWKKSGDFMTIAGFNAENEILHTLAFLAGLMIWSQ 120
PPI-BnCPP               HFHFVHEFVTILMDSAILFFGILPWFWKISGGFMPMVGLDFENEILHTLSFLAGLMIWSQ 120
PPI-SoyCPP              HFHFVHEFVTIVTDSTILYFGVLPWFWKKSGDFMTIAGFNAENEILHTLAFLAGLMIWSQ 120
Consensus PPI           HFHFVHEFVTIXXDSXILXFGXLPWFWKXSGXFXXXXGXXXENEILHTLXFLAGLMXWSQ 120

                        130       140       150       160       170       180
                        |....|....|....|....|....|....|....|....|....|....|....|
PPI-AtCPP               ITDLPFSLYSTFVIEARHGFNKQTPWLFFRDMLKGIFLSVIIGPPIVAAIIVIVQKGGPY 180
PPI-BnCPP               ITDLPFSLYSTFVIESRHGFNKQTIWMFIRDMIKGILLSVIPAPPIVAAIIVIVQKGGPY 180
PPI-SoyCPP              ITDLPFSLYSTFVIEARHGFNKQTPWLFFRDMLKGIFLSVIIGPPIVAAIIVIVQKGGPY 180
Consensus PPI           ITDLPFSLYSTFVIEXRHGFNKQTXWXFXRDMXKGIXLSVIXXPPIVAAIIVIVQKGGPY 180

                        190       200       210       220       230       240
                        |....|....|....|....|....|....|....|....|....|....|....|
PPI-AtCPP               LAIYLWVFTFGLSIVMMTLYPVLIAPLFNKFTPLPDGQLREKIEKLASSLNYPLKKLFVV 240
PPI-BnCPP               LAIYLWAFMFILSIVMMTIYPVLIAPLFNKFTPLPDGDLREKIEKLASSLKFPLKKLFVV 240
PPI-SoyCPP              LAIYLWVFTFGLSIVMMTLYPVLIAPLFNKFTPLPDGQLREKIEKLASSLNYPLKKLFVV 240
Consensus PPI           LAIYLWXFXFXLSXVMMTXYPVLIAPLFNKFTPLPDGXLREKIEKLASSLXXPLKKLFVV 240

                        250       260       270       280       290       300
                        |....|....|....|....|....|....|....|....|....|....|....|
PPI-AtCPP               DGSTRSSHSNAYMYGFFKNKRIVPYDTLIQQCKDDEEIVAVIAHELGHWKLNHTVYTFVA 300
PPI-BnCPP               DGSTRSSHSNAYMYGFFKNKRIVLYDTLIQQCQNENEIVAVIAHELGHWKLNHTTYSFIA 300
PPI-SoyCPP              DGSTRSSHSNAYMYGFFKNKRIVPYDTLIQQCKDDEEIVAVIAHELGHWKLNHTVYTFVA 300
Consensus PPI           DGSTRSSHSNAYMYGFFKNKRIVXYDTLIQQCXXXXEIVAVIAHELGHWKLNHTXYXFXA 300

                        310       320       330       340       350       360
                        |....|....|....|....|....|....|....|....|....|....|....|
PPI-AtCPP               MQILTLLQFGGYTLVRNSADLYRSFGFDTQPVLIGLIIFQHTVIPLQQLVSFGLNLVSRS 360
PPI-BnCPP               VQILAFLQFGGYTLVRNSTDLFRSFGFDTQPVLIGLIIFQHTVIPLQHLVSFDLNLVSRA 360
PPI-SoyCPP              MQILTLLQFGGYTLVRNSADLYRSFGFDTQPVLIGLIIFQHTVIPLQQLVSFGLNLVSRS 360
Consensus PPI           XQILXXLQFGGYTLVRNSXDLXRSFGFDTQPVLIGLIIFQHTVIPLQXLVSFXLNLVSRX 360

                        370       380       390       400       410       420
                        |....|....|....|....|....|....|....|....|....|....|....|
PPI-AtCPP               FEFQADGFAKKLGYASGLRGGLVKLQEENLSAMNTDPWYSAYHYSHPPLVERLAALDEPD 420
PPI-BnCPP               FEFQADAFAVNLGYAKDLRPALVKLQEENLSAMNTDPLYSAYHYSHPPLVERLRAIDGED 420
PPI-SoyCPP              FEFQADGFAKKLGYASGLRGGLVKLQEENLSAMNTDPWYSAYHYSHPPLVERLAALDEPD 420
Consensus PPI           FEFQADXFAXXLGYAXXLRXXLVKLQEENLSAMNTDPXYSAYHYSHPPLVERLXAXDXXD 420

                        430       440       450       460       470       480
                        |....|....|....|....|....|....|....|....|....|....|....|
PPI-AtCPP               KKED------------------------------------------------------ 424
PPI-BnCPP               KKTD------------------------------------------------------ 424
PPI-SoyCPP              KKED------------------------------------------------------ 424
Consensus PPI           KKXD                                                        480
```

Table 37. ClustalW Analysis of PPI/Generic Nucleic Acids

1) **PPI-AtCPP** (SEQ ID NO:97)
2) **PPI-BnCPP** (SEQ ID NO:109)
3) **PPI-SoyCPP** (SEQ ID NO:112)
4) **afc1** (SEQ ID NO:124)
5) **AT4g01320** (SEQ ID NO:126)
6) **AF007269** (SEQ ID NO:128)
6) **Consensus** (SEQ ID NO:170)

```
                        10        20        30        40        50        60        70
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
PPI-AtCPP NA    ------------------------------------------------------------------------
PPI-BnCPP       ------------------------------------------------------------------------
PPI-SoyCPP      ------------------------------------------------------------------------
afc1            ------------------------------------------------------------------------
AT4g01320       ------------------------------------------------------------------------
AF007269        ATGGCGATTCCTTTCATGGAAACCGTCGTGGGTAAGCTTCAAAACCTTTTTCTGAGACATTTTACTATCC
Consensus       ------------------------------------------------------------------------

                        80        90       100       110       120       130       140
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
PPI-AtCPP NA    ----------ATGGCGTTTCCCTACATGGAAGCCGTTGTCGGATTTATGATATTAATGTACATTTTTGAA
PPI-BnCPP       ------------------------------------------------------------------------
PPI-SoyCPP      ----------ATGGCGTTTCCCTACATGGAAGCCGTTGTCGGATTTATGATATTAATGTACATTTTTGAA
afc1            ------------------------------------------------------------------------
AT4g01320       ------------------------------------------------------------------------
AF007269        TGTTTCACTCATCGTATTTCGTTTTTGTTTGGGTTTTGCTTTCTGTGTTGTGTGTGTTGAGATTCCATGA
Consensus       ----------ATGGCGATTCCTTTCATGGAAACCGTCGT-GGTTTTATGATAT--ATGTACATTTTTGAA

                       150       160       170       180       190       200       210
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
PPI-AtCPP NA    ACTTACTTGGATG-TGCGACAACATAGGGCCCTCAAACTTCCTACTCTTCCAAAGACTTTAGAGGGTGTT
PPI-BnCPP       ------------------------------------------------------------------------
PPI-SoyCPP      ACTTACTTGGATG-TGCGACAACATAGGGCCCTCAAACTTCCTACTCTTCCAAAGACTTTAGAGGGTGTT
afc1            ------------------------------------------------------------------------
AT4g01320       ------------------------------------------------------------------------
AF007269        -CTCGTTTGTTTCATATACCATCGTCTCTGCTTCTCGTTTCTAAATTTTGTTCTTTTCTAATAGTGCGTA
Consensus       --CTATTTGGAT----TGGCAACATG----CCTCAA--CTTCCACTCTCC---AAACTTGGTGGTGTAT-

                       220       230       240       250       260       270       280
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
PPI-AtCPP NA    ATCAGCCAAGAGAAATTTGAGAAATCTAGAGCCTATAGTCTTGATAAAAGCCACTTCCATTTTGTTCACG
PPI-BnCPP       ------------------------------------------------------------------------
PPI-SoyCPP      ATCAGCCAAGAGAAATTTGAGAAATCTAGAGCCTATAGTCTTGATAAAAGCCACTTCCATTTTGTTCACG
afc1            ------------------------------------------------------------------------
AT4g01320       ------------------------------------------------------------------------
AF007269        CCTTGATCTGAGGTTTTATTACTCCTACTAGTTTCTTGTCTTACTCGTG--CGTTT-GATTTGATTTGAG
Consensus       ---AGCCAAGAGAAGTTTGAGAAATCTGAG--CTACAGTCTTGAAAAAG--CATT--CATTT-GTTCA-G

                       290       300       310       320       330       340       350
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
PPI-AtCPP NA    AGTTTGTGACAATAGTGACAGACTCTACAATTTTGTACTTTGGGGTATTGCCCTGGTTTTGGAA-----G
PPI-BnCPP       ------------------------------------------------------------------------
PPI-SoyCPP      AGTTTGTGACAATAGTGACAGACTCTACAATTTTGTACTTTGGGGTATTGCCCTGGTTTTGGAA-----G
afc1            ------------------------------------------------------------------------
AT4g01320       ------------------------------------------------------------------------
AF007269        CTTATGTGA-TTTCATCATCTCTTCCTCGGTTTTAGAATGTACGGAGCTTCTCTGTTAACCAAAATCTAG
Consensus       AGTTTGTA--CATAGT--TAGACTCT-CAATTTTGT-CTTTGGG---TTTGCCTGGTTTTGGAA-----G

                       360       370       380       390       400       410       420
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
PPI-AtCPP NA    AAATCAGGAGATTTTATGACAATAGCTGGTTTCAATGCTGAGAATGAAATACTGCATACCCTTGCCTTCT
PPI-BnCPP       ------------------------------------------------------------------------
PPI-SoyCPP      AAATCAGGAGATTTTATGACAATAGCTGGTTTCAATGCTGAGAATGAAATACTGCATACCCTTGCCTTCT
afc1            ------------------------------------------------------------------------
AT4g01320       ------------------------------------------------------------------------
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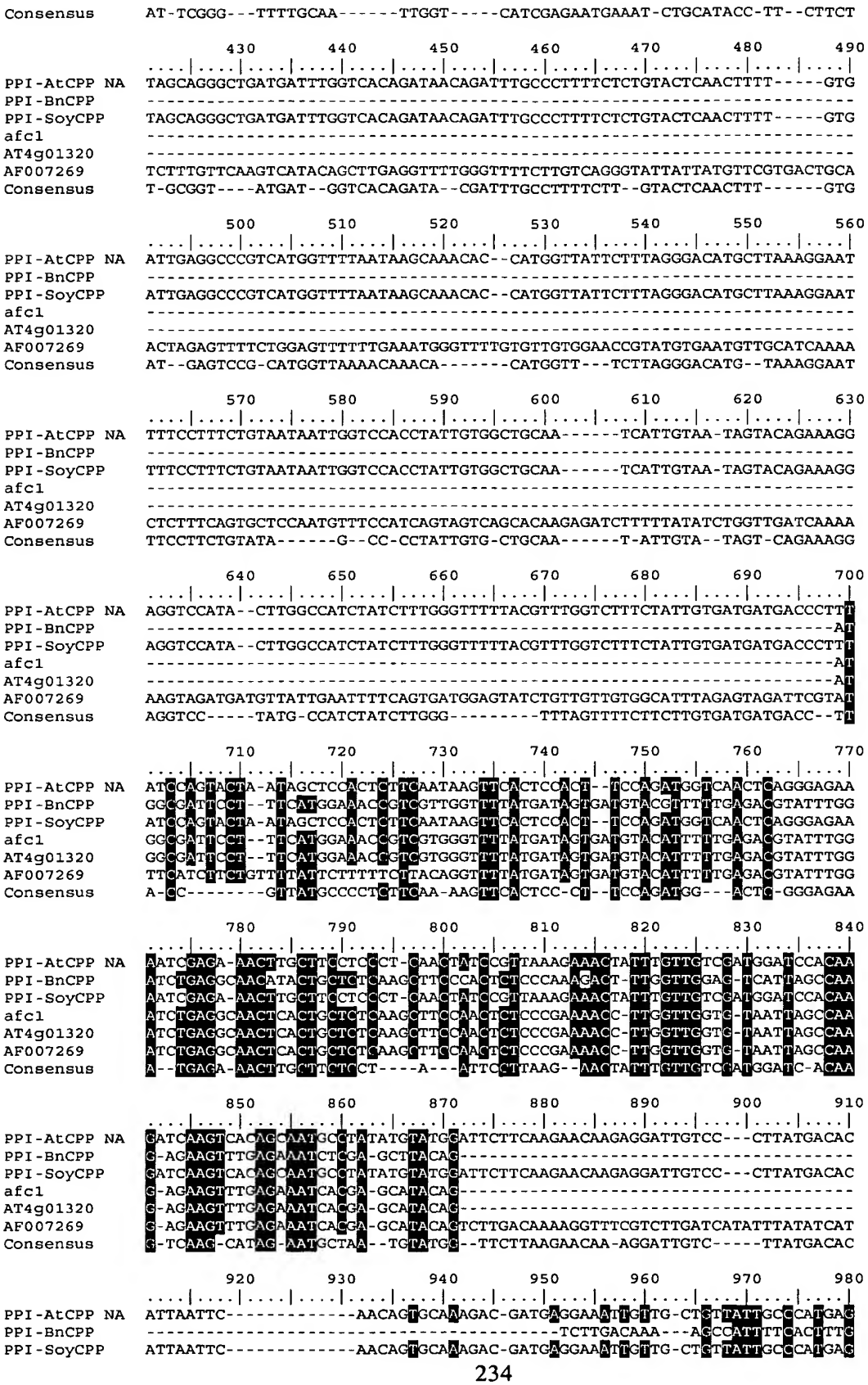
Consensus AT-TCGGG---TTTTGCAA------TTGGT-----CATCGAGAATGAAAT-CTGCATACC-TT--CTTCT
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PPI-AtCPP NA TAGCAGGGCTGATGATTTGGTCACAGATAACAGATTTGCCCTTTTCTCTGTACTCAACTTTT-----GTG
PPI-BnCPP
PPI-SoyCPP TAGCAGGGCTGATGATTTGGTCACAGATAACAGATTTGCCCTTTTCTCTGTACTCAACTTTT-----GTG
afc1
AT4g01320
AF007269 TCTTTGTTCAAGTCATACAGCTTGAGGTTTTGGGTTTTCTTGTCAGGGTATTATTATGTTCGTGACTGCA
Consensus T-GCGGT----ATGAT--GGTCACAGATA--CGATTTGCCTTTTCTT--GTACTCAACTTT------GTG
500 510 520 530 540 550 560
PPI-AtCPP NA ATTGAGGCCCGTCATGGTTTTAATAAGCAAACAC--CATGGTTATTCTTTAGGGACATGCTTAAAGGAAT
PPI-BnCPP
PPI-SoyCPP ATTGAGGCCCGTCATGGTTTTAATAAGCAAACAC--CATGGTTATTCTTTAGGGACATGCTTAAAGGAAT
afc1
AT4g01320
AF007269 ACTAGAGTTTTCTGGAGTTTTTTGAAATGGGTTTTGTGTTGTGGAACCGTATGTGAATGTTGCATCAAAA
Consensus AT--GAGTCCG-CATGGTTAAAACAAACA-------CATGGTT---TCTTAGGGACATG--TAAAGGAAT
570 580 590 600 610 620 630
PPI-AtCPP NA TTTCCTTTCTGTAATAATTGGTCCACCTATTGTGGCTGCAA------TCATTGTAA-TAGTACAGAAAGG
PPI-BnCPP
PPI-SoyCPP TTTCCTTTCTGTAATAATTGGTCCACCTATTGTGGCTGCAA------TCATTGTAA-TAGTACAGAAAGG
afc1
AT4g01320
AF007269 CTCTTTCAGTGCTCCAATGTTTCCATCAGTAGTCAGCACAAGAGATCTTTTTATATCTGGTTGATCAAAA
Consensus TTCCTTCTGTATA------G--CC-CCTATTGTG-CTGCAA------T-ATTGTA--TAGT-CAGAAAGG
640 650 660 670 680 690 700
PPI-AtCPP NA AGGTCCATA--CTTGGCCATCTATCTTTGGGTTTTTACGTTTGGTCTTTCTATTGTGATGATGACCCTTT
PPI-BnCPP AT
PPI-SoyCPP AGGTCCATA--CTTGGCCATCTATCTTTGGGTTTTTACGTTTGGTCTTTCTATTGTGATGATGACCCTTT
afc1 AT
AT4g01320 AT
AF007269 AAGTAGATGATGTTATTGAATTTTCAGTGATGGAGTATCTGTTGTTGTGGCATTTAGAGTAGATTCGTAT
Consensus AGGTCC-----TATG-CCATCTATCTTGGG---------TTTAGTTTTCTTCTTGTGATGATGACC--TT
710 720 730 740 750 760 770
PPI-AtCPP NA ATCCAGTACTA-ATAGCTCCACTCTTCAATAAGTTCACTCCACT--TCCAGATGGTCAACTCAGGGAGAA
PPI-BnCPP GGCCATTCCT--TTCATGGAAACCGTCGTTGGTTTTATGATAGTGATGTACGTTTTTGAGACGTATTTGG
PPI-SoyCPP ATCCAGTACTA-ATAGCTCCACTCTTCAATAAGTTCACTCCACT--TCCAGATGGTCAACTCAGGGAGAA
afc1 GGCCATTCCT--TTCATGGAAACCGTCGTGGGTTTTATGATAGTGATGTACATTTTTGAGACGTATTTGG
AT4g01320 GGGGATTCCT--TTCATGGAAACCGTCGTGGGTTTTATGATAGTGATGTACATTTTTGAGACGTATTTGG
AF007269 TTCATCTTCTGTTTTATTCTTTTTCTTACAGGTTTTATGATAGTGATGTACATTTTTGAGACGTATTTGG
Consensus A-CC--------GTTATGCCCCTCTTCAA-AAGTTCACTCC-CT--TCCAGATGG---ACTC-GGGAGAA
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PPI-AtCPP NA AATCGAGA-AACTTGCTTCTCCCT-CAACTATCCGTTAAAGAAACTATTTGTTGTCGATGGATCCACAA
PPI-BnCPP ATCTGAGGCAACATACTGCTCTCAAGCTTCCCACTCTCCCAAACACT-TTGGTTGGAG-TCATTAGCCAA
PPI-SoyCPP AATCGAGA-AACTTGCTTCTCCCT-CAACTATCCGTTAAAGAAACTATTTGTTGTCGATGGATCCACAA
afc1 ATCTGAGGCAACTCACTGCTCTCAAGCTTCCAACTCTCCCGAAAACC-TTGGTTGGTG-TAATTAGCCAA
AT4g01320 ATCTGAGGCAACTCACTGCTCTCAAGCTTCCAACTCTCCCGAAAACC-TTGGTTGGTG-TAATTAGCCAA
AF007269 ATCTGAGGCAACTCACTGCTCTCAAGCTTCCAACTCTCCCGAAAACC-TTGGTTGGTG-TAATTAGCCAA
Consensus A--TGAGA-AACTTGCTTCTCCT----A---ATTCCTTAAG--AACTATTTGTTGTCGATGGATC-ACAA
850 860 870 880 890 900 910
PPI-AtCPP NA GATCAAGTCACAGCAATGCCTATATGTATGGATTCTTCAAGAACAAGAGGATTGTCC---CTTATGACAC
PPI-BnCPP G-AGAAGTTTGAGAAATCTCGA-GCTTACAG
PPI-SoyCPP GATCAAGTCACAGCAATGCCTATATGTATGGATTCTTCAAGAACAAGAGGATTGTCC---CTTATGACAC
afc1 G-AGAAGTTTGAGAAATCACGA-GCATACAG
AT4g01320 G-AGAAGTTTGAGAAATCACGA-GCATACAG
AF007269 G-AGAAGTTTGAGAAATCACGA-GCATACAGTCTTGACAAAAGGTTTCGTCTTGATCATATTTATATCAT
Consensus G-TCAAG-CATAG-AATGCTAA--TGTATGG--TTCTTAAGAACAA-AGGATTGTC-----TTATGACAC
920 930 940 950 960 970 980
PPI-AtCPP NA ATTAATTC----------AACAGTGCAAAGAC-GATGAGGAAATTGTTG-CTGTTATTGCCCATGAG
PPI-BnCPP TCTTGACAAA---AGCCATTTTCACTTTG
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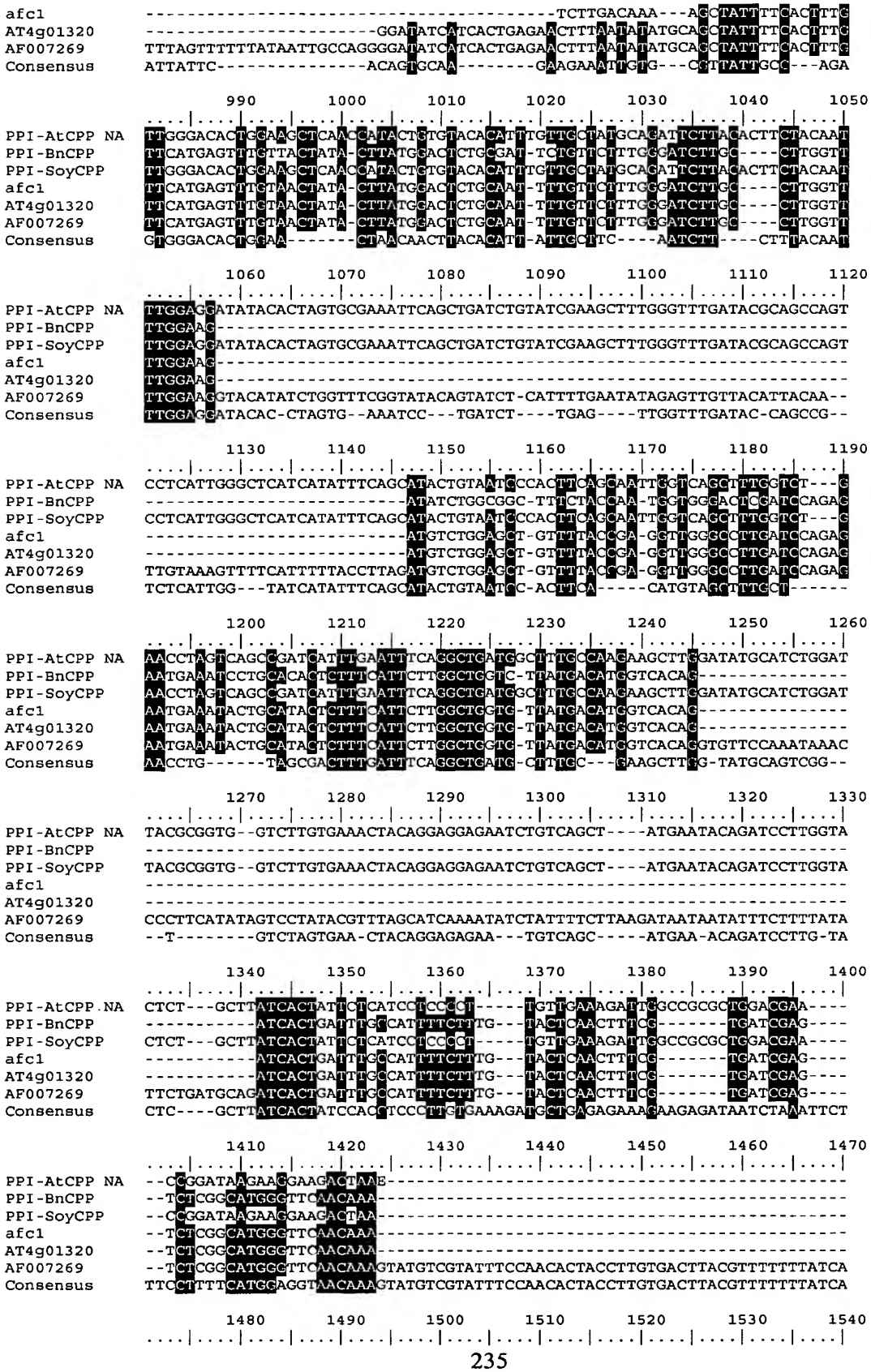
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PPI-BnCPP
PPI-SoyCPP
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1000
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1020
1030
1040
1050
1060
1070
1080
1090
1100
1110
1120
1130
1140
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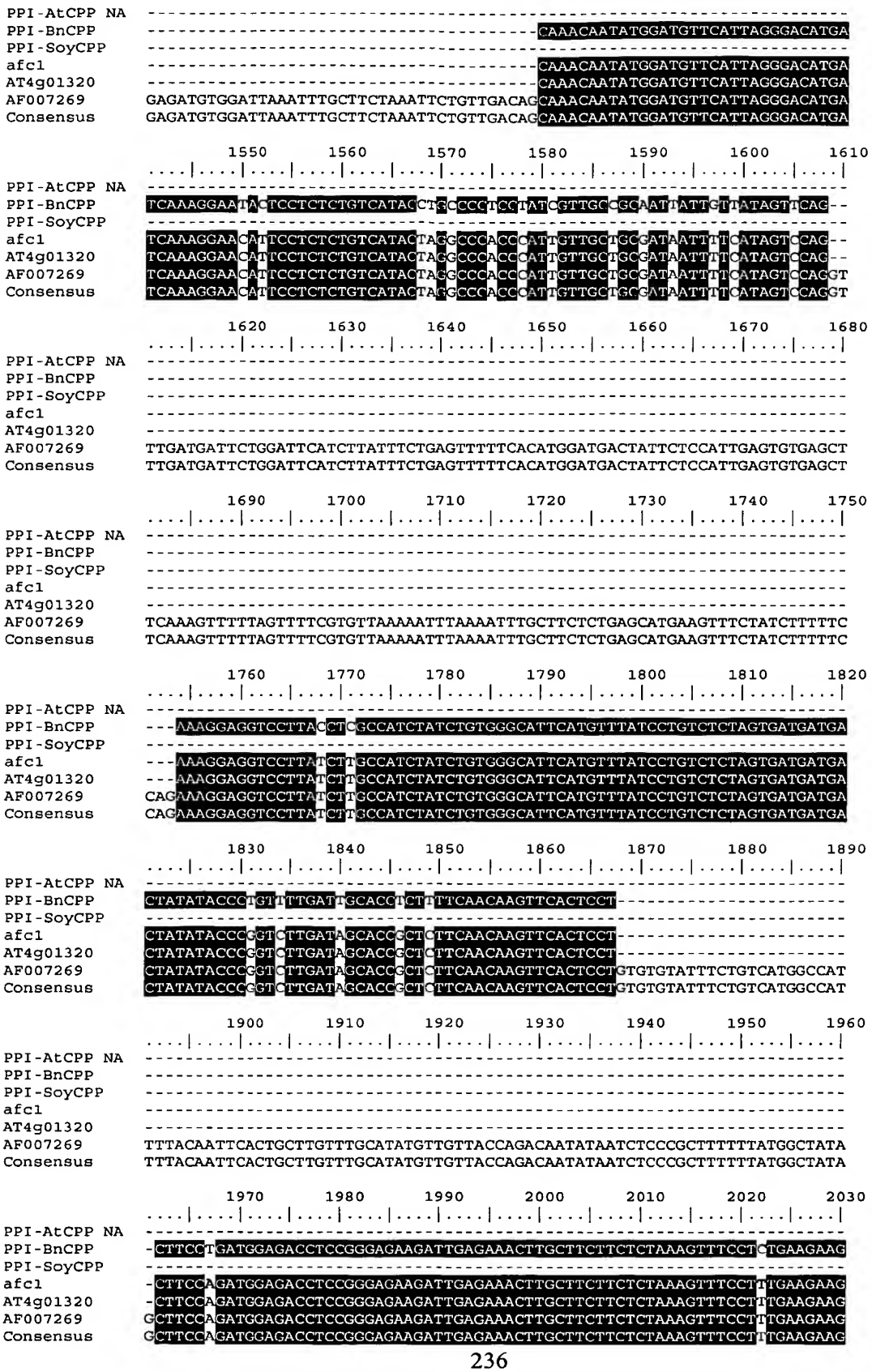
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PPI-BnCPP
PPI-SoyCPP
afc1
AT4g01320
AF007269
Consensus
1550 1560 1570 1580 1590 1600 1610
1620 1630 1640 1650 1660 1670 1680
1690 1700 1710 1720 1730 1740 1750
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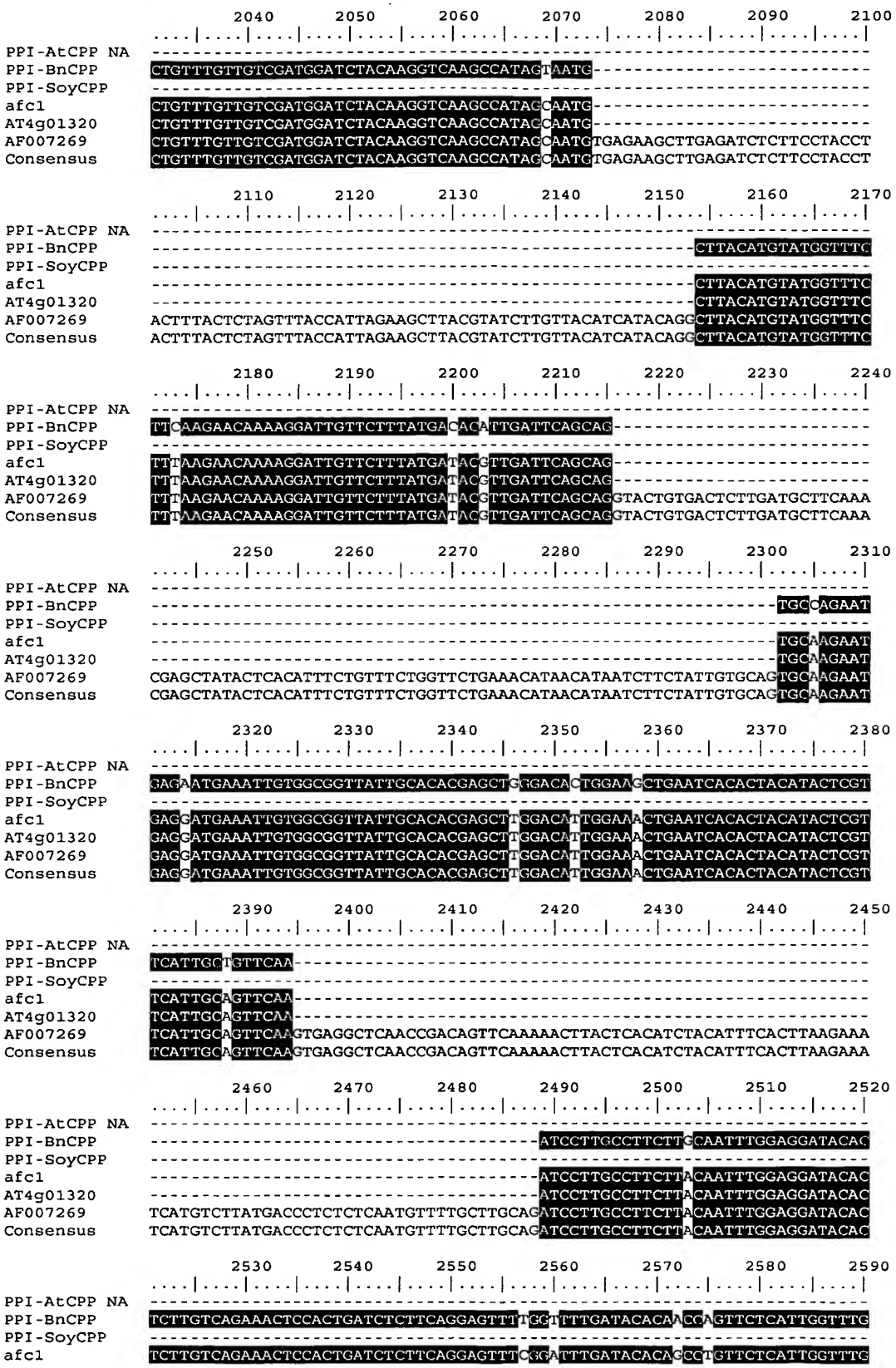
2040 2050 2060 2070 2080 2090 2100
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PPI-SoyCPP
afc1 CTGTTTGTTGTCGATGGATCTACAAGGTCAAGCCATAGCAATG
AT4g01320 CTGTTTGTTGTCGATGGATCTACAAGGTCAAGCCATACCAATG
AF007269 CTGTTTGTTGTCGATGGATCTACAAGGTCAAGCCATAGCAATGTGAGAAGCTTGAGATCTCTTCCTACCT
Consensus CTGTTTGTTGTCGATGGATCTACAAGGTCAAGCCATAGCAATGTGAGAAGCTTGAGATCTCTTCCTACCT
2110 2120 2130 2140 2150 2160 2170
PPI-AtCPP NA
PPI-BnCPP CTTACATGTATGGTTTC
PPI-SoyCPP
afc1 CTTACATGTATGGTTTC
AT4g01320 CTTACATGTATGGTTTC
AF007269 ACTTTACTCTAGTTTACCATTAGAAGCTTACGTATCTTGTTACATCATACAGGCTTACATGTATGGTTTC
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2180 2190 2200 2210 2220 2230 2240
PPI-AtCPP NA
PPI-BnCPP TTCAAGAACAAAAGGATTGTTCTTTATGACAGATTGATTCAGCAG
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2250 2260 2270 2280 2290 2300 2310
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PPI-BnCPP TGCCAGAAT
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2320 2330 2340 2350 2360 2370 2380
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PPI-BnCPP GAGAATGAAATTGTGGCGGTTATTGCACACGAGCTGGGACACTGGAAGCTGAATCACACTACATACTCGT
PPI-SoyCPP
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AF007269 GAGGATGAAATTGTGGCGGTTATTGCACACGAGCTTGGACATTGGAAACTGAATCACACTACATACTCGT
Consensus GAGGATGAAATTGTGGCGGTTATTGCACACGAGCTTGGACATTGGAAACTGAATCACACTACATACTCGT
2390 2400 2410 2420 2430 2440 2450
PPI-AtCPP NA
PPI-BnCPP TCATTGCTGTTCAA
PPI-SoyCPP
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AT4g01320 TCATTGCAGTTCAA
AF007269 TCATTGCAGTTCAAGTGAGGCTCAACCGACAGTTCAAAAACTTACTCACATCTACATTTCACTTAAGAAA
Consensus TCATTGCAGTTCAAGTGAGGCTCAACCGACAGTTCAAAAACTTACTCACATCTACATTTCACTTAAGAAA
2460 2470 2480 2490 2500 2510 2520
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PPI-BnCPP ATCCTTGCCTTCTTGCAATTTGGAGGATACAC
PPI-SoyCPP
afc1 ATCCTTGCCTTCTTACAATTTGGAGGATACAC
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Consensus TCATGTCTTATGACCCTCTCTCAATGTTTTGCTTGCAGATCCTTGCCTTCTTACAATTTGGAGGATACAC
2530 2540 2550 2560 2570 2580 2590
PPI-AtCPP NA
PPI-BnCPP TCTTGTCAGAAACTCCACTGATCTCTTCAGGAGTTTTGGTTTTGATACACAACGAGTTCTCATTGGTTTG
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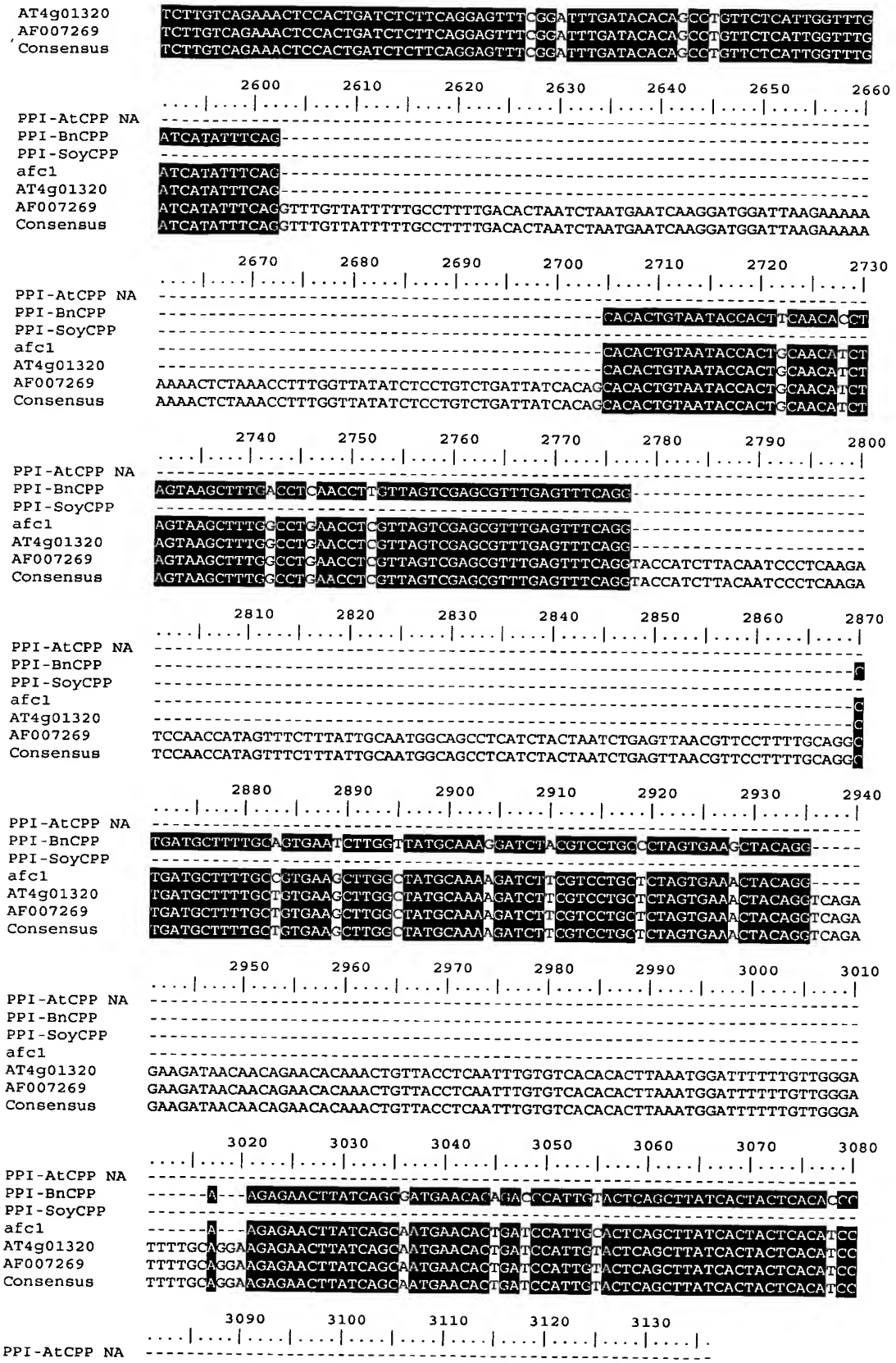
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Consensus
2670 2680 2690 2700 2710 2720 2730
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PPI-SoyCPP
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AT4g01320
AF007269
Consensus
2740 2750 2760 2770 2780 2790 2800
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Consensus
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PPI-SoyCPP
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AT4g01320
AF007269
Consensus
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PPI-BnCPP
PPI-SoyCPP
afc1
AT4g01320
AF007269
Consensus
3020 3030 3040 3050 3060 3070 3080
PPI-AtCPP NA
PPI-BnCPP
PPI-SoyCPP
afc1
AT4g01320
AF007269
Consensus
3090 3100 3110 3120 3130
PPI-AtCPP NA

Table XX. ClustalW Analysis of PPI/Generic Nucleic Acids

1) **PPI-AtCPP** (SEQ ID NO:97)
2) **PPI-BnCPP** (SEQ ID NO:109)
3) **PPI-SoyCPP** (SEQ ID NO:112)
4) **afc1** (SEQ ID NO:124)
5) **AT4g01320** (SEQ ID NO:126)
6) **AF007269** (SEQ ID NO:128)
6) **Consensus** (SEQ ID NO:170)

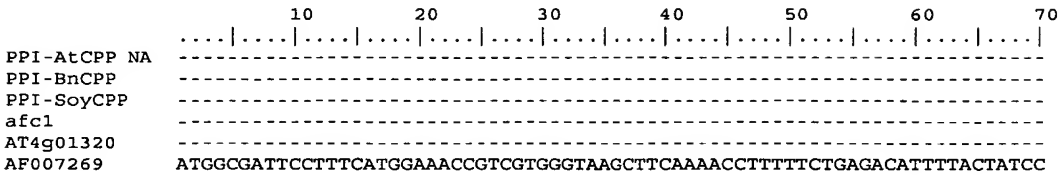
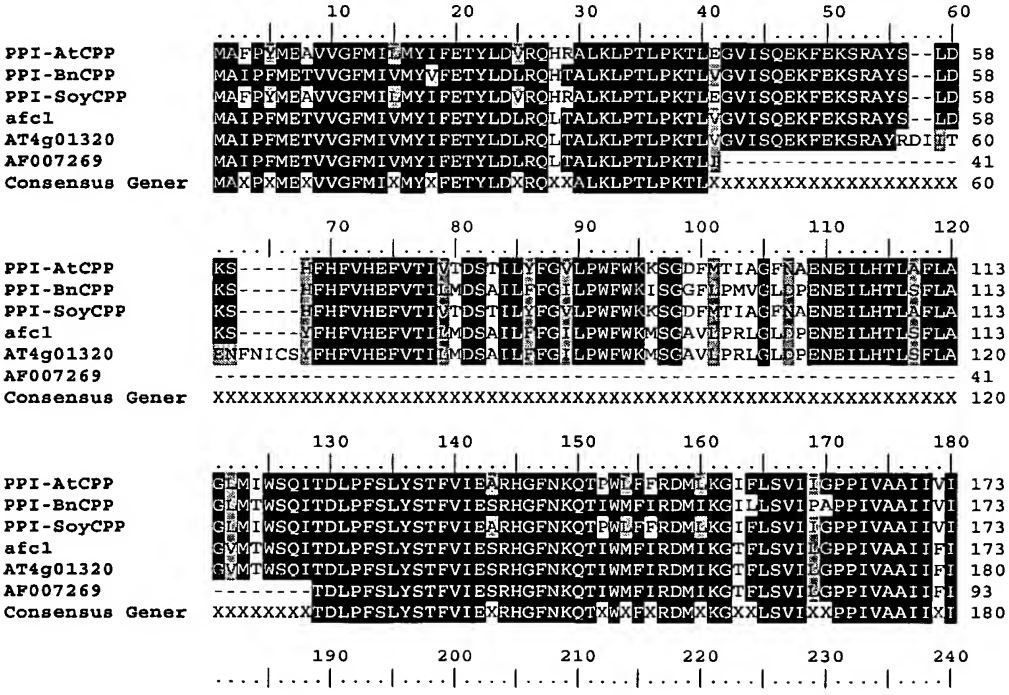
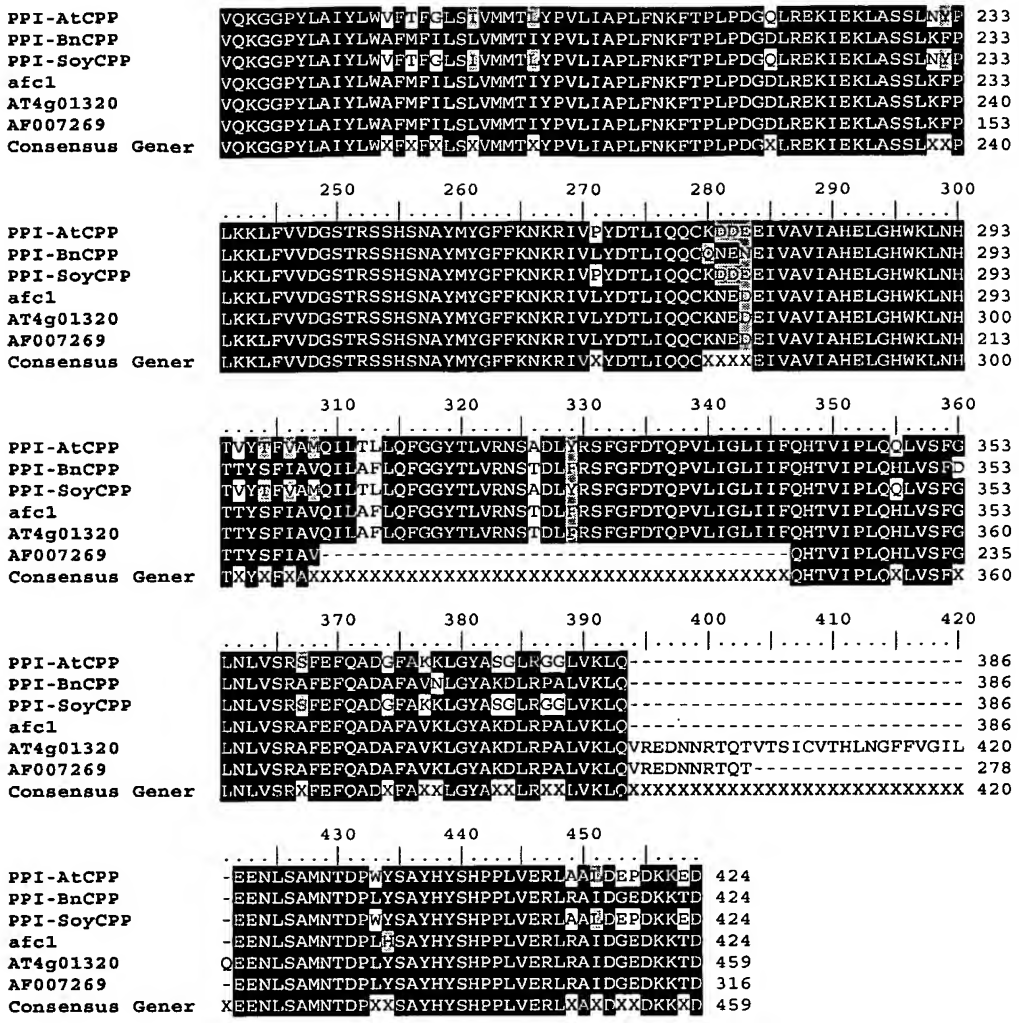


Table 38. ClustalW Analysis of PPI/Generic Amino Acids

1) **PPI-AtCPP** (SEQ ID NO:98)
2) **PPI-BnCPP** (SEQ ID NO:110)
3) **PPI-SoyCPP** (SEQ ID NO:113)
4) **afc1** (SEQ ID NO:125)
5) **AT4g01320** (SEQ ID NO:127)
6) **AF007269** (SEQ ID NO:129)
7) **Consensus Gener** (SEQ ID NO:169)





Example 47 Cloning, vector construction and over-expression of AtFT-B sequences in Arabidopsis produces a dominant-negative phenotype

Farnesyltransferase is a heterodimer formed by its α- and β- subunits and its activity relies on the proper dimerization between these subunits. Increased ABA sensitivity can be achieved by the over-expression of a non-full-length form of AtFTB (SEQ ID NO:1) in Arabidopsis. In the corollary experiment, over-expression of the full-length AtFTB failed to alter the ABA sensitivity. These results suggest that the phenotype of enhanced ABA response is likely the result of dominant-negative effect of the truncated form AtFTB. The truncated AtFTB maybe nonfunctional or possess limited functionality *in vivo* as compared to a full length endogenous subunit. However. The reduction of Ft activity results in enhanced ABA sensitivity.

Cloning

The farnesyltransferase sequence described by SEQ ID NO:1 was cloned into an appropriate vector under the transcriptional control of the 35S CaMV promoter (pBI121 derived vector) in the sense orientation for expression in plant cells. This vector was designated ΔN90AtFTB and designated SEQ ID NO:79. The protein encoded by SEQ ID NO:1 has been determined to lack the 5' 270 nucleotides, and therefore does not code for the 5' terminal 90 amino acids. The full length farnesyltransferase sequence was obtained using the primer pair identified by SEQ ID NO:86 and SEQ ID NO:171 and methodology as described elsewhere in this document. The resulting sequence, identified as SEQ ID NO:172 was cloned into an appropriate vector under the transcriptional control of the 35S CaMV promoter (pBI121 derived vector) in the sense orientation for expression in plant cells. This vector was designated pBI121-AtFTB, SEQ ID NO:173. The protein encoded by SEQ ID NO:172 has been determined to represent the full length polypeptide.

Agrobacterium-mediated transformation, transgenic line selection and ABA test. Agrobacterium strain GV3101 carrying the binary constructs described above were transformed into Arabidopsis thaliana via agrobacterium-mediated floral dipping transformation. Transformed Arabidopsis lines (T1) were selected on Murashige/Skoog (Sigma) plates containing kanamycin (50 μg/μl). Kanamycin-resistant seedlings were then transferred to soil. The subsequent T2 seeds were harvested from individual transgenic lines for ABA tests.

Northern blot analysis. Total RNA was isolated from two-week-old T2 Arabidopsis plants of the pBI121-ΔN90AtFTB, as well as from wild-type Columbia and *era*1 mutant plants. After separated in the agarose gel, RNA was transferred onto the nitrocellulose membrane and was hybridized with the ^{32}P-labelled ΔN90AtFTB DNA probe.

Over-expression of pBI121-ΔN90AtFTB, not pBI121-AtFTB resulted in enhanced ABA sensitivity:

Transgenic plants were selected and advanced to the second generation. T2 seeds of these two constructs were subjected to ABA test using 0.0, 0.25, 0.5 and 1.0 μM ABA in minimum MS-agarose plates. Of the fifteen pBI121-ΔN90AtFTB lines ten showed an enhanced ABA sensitivity phenotype. At 0.5 μM ABA, the seeds would germinate, however, the

development of the seedlings for these 10 lines were retarded or arrested, showing a typical ABA hypersensitive response. In contrast, of the fifteen pBI121-AtFTB transgenic lines, all but one line showed normal wild-type like ABA response to seed germination and seedling development.

Northern blot analysis indicated that in the transgenic lines of pBI121-ΔN90AtFTB, the expression levels were higher than the endogenous AtFTB transcript level as depicted by the wild-type control. This indicates the ABA hypersensitive phenotype of these transgenic lines is unlikely due to transcriptional co-suppression. The enhanced ABA response correlates with the results of other methods of AtFTB down-regulation, such as anti-sense and RNAi, hairpin constructs. It is possible that the observed ABA hypersensitive response in ΔN90AtFTB transgenic lines are due to a dominant negative effect. The high transcript levels of ΔN90AtFTB should produce an abundance of the truncated form of AtFTB which may bind to the endogenous AtFTA and result in competitive inhibition of AtFTase activity.

Further support for the interaction of truncated FT-B with endogenous FT-A comes from a yeast two-hybrid interaction experiment. Use of the ΔN90AtFTB cDNA as bait, identified interacting clones the majority of which were found to encode FT-A.

SEQ ID NO:79 **pBI121-ΔN90AtFTB Truncated FT-B Vector**

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caatgtgttattaagttgtctaagcgtcaatttgtttacaccacaatatatcctgcca

SEQ ID NO:86 FORWARD Primer SacI site 5' aaaCCCGGGatgccagtagtaacccgc 3'
SEQ ID NO:171 REV Primer BamHI site 5' aaaggatcctcatgctgctttaaagaagaactcgat 3'

SEQ ID NO:172 Full length FT-B
cccgggatgccagtagtaacccgcttgattcgtttgaagtgtgtagggctcagacttgaccggagtggactcaatcg
gcgaatctgtcacggaggacacggggaatcaacgcggcggagagtgatggaagagctttcaagcctaaccgtgagtc
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SEQ ID NO:177 Full Length FT-B amino acid sequence encoded by SEQ ID NO:172
MPVVTRLIRLKCVGLRLDRSGLNRRICHGGHGESTRRRVMEELSSLTVSQREQFLVENDVFGIYNYFDASDVSTQKY
MMEIQRDKQLDYLMKGLRQLGPQFSSLDANRPWLCYWILHSIALLGETVDDELESNAIDFLGRCQGSEGGYGGGPGQ
LPHLATTYAAVNALVTLGGDKALSSINREKMSCFLRRMKDTSGGFRMHDMGEMDVRACYTAISVASILNIMDDELTQ
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LHNIVMDQYNEAIEFFFKAA

SEQ ID NO:173 **pBI121-AtFTB (Full length vector Over-expression)**

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ttgtttacaccacaatatatcctgcca

EXAMPLE 48 Cloning and transformation of isoprenylcysteine carboxyl methyltransferase

The *Arabidopsis* isoprenylcysteine carboxyl methyltransferase (ICMT) sequence was obtained by RT-PCR amplification using the protocol described above. The sequence was produced using the primer pair identified by SEQ ID NO:174 (5'-aaaggatccatgacagagatcttcagtgacacca-3') and SEQ ID NO:175 (5'-aaagagctctcagttcacaaatggaacaccaga-3'). The sequence is identical to that reported by Accession number AB007648, GI:10177821 (Dec. 2000).

The isolated sequence was used to generate plant transformation vectors designed either to express the encoded protein or down-regulate expression. The vectors were used to transform Arabidopsis by the flower dipping method described elsewhere. Transformed plants were selected and propagated. Molecular and physiological analysis of the transgenic lines can be performed as detailed in other examples. Such analysis can include; molecular studies such as PCR, Southern, Northern and Western analysis; physiological analysis such as; growth studies,

tolerance to environmental stress (drought, salt, heat, cold,) tolerance to biotic stress, nutritional stress, as well as biochemical analysis.

SEQ ID NO:176
atgacagagatcttcagtgacaccagcatcagacagttatctcaaatgctactatcactaatcttcttccacatatccgaatacattctagccatc
accattcacggagcatcaaacgtaactcttagttcgcttttaatcaccaagcattacgctttagcaatgcttctgtcgcttctcgaatacctaacg
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gcctctggtgttccatttgtgaactga

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All citations in this application to materials and methods are hereby incorporated by reference.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described specifically herein. Such equivalents are intended to be encompassed in the scope of the following claims.

What is claimed is:

1. A method of producing a transgenic plant, wherein said plant has an altered phenotype selected from the group consisting of increased tolerance to stress, delayed senescence, increased ABA sensitivity, increased yield, increased productivity and increased biomass compared to a wild type plant, comprising: introducing into a plant cell a compound that inhibits farnesylation of a polypeptide having a carboxyl terminal CaaX motif to generate a transgenic cell; and regenerating a transgenic plant from said transgenic cell.

2. The method of claim 1, wherein said compound inhibits farnesyltransferase, prenylprotease, or prenylcysteine carboxyl methyltransferase expression or activity.

3. The method of claim 1, wherein said compound comprises an antisense nucleic acid sequence encoding farnesyltransferase or a portion thereof, operably linked to a promoter that is active in said plant cell.

4. The method of claim 3, wherein said promoter is selected from the group consisting of a constitutive promoter, an ABA inducible promoter, tissue specific promoters or a guard cell-specific promoter.

5. The method of claim 3, wherein said antisense nucleic acid comprises 20 or more consecutive nucleic acids complementary to any one of SEQ ID NOs: 1, 14, 40, 43, 80-85 or 172.

6. The method of claim 3, wherein said antisense nucleic acid comprises any one of SEQ ID NOs: 36, 41, or 44.

7. The method of claim 1, wherein said compound is a nucleic acid is selected from the group consisting of SEQ ID NO: 54-64.

8. The method of claim 1, wherein said compound comprises a nucleic acid molecule comprising a nucleic acid sequence encoding a mutated farnesyl transferase beta polypeptide or a fragment thereof.

9. The method of claim 1, wherein said compound comprises any one of SEQ ID NOs: 1, 14, 40, 43, 80-85, 172 or a fragment thereof.

10. The method of claim 8, wherein said nucleic acid encodes a polypeptide which is less than 314 amino acids in length.

11. The method of claim 8, wherein the nucleic acid molecule encodes a polypeptide capable of forming a dimer.

12 The method of clam 11, wherein said dimer is a heterodimer.

13. The method of claim 1, wherein said compound comprises a nucleic acid encoding a CaaX motif operably linked to a promoter.

14 The transgenic plant produced the method of claim 1.

15. The seed produced by the transgenic plant of claim 14, wherein said seed produces a plant that has an altered phenotype selected from the group consisting of increased tolerance to stress, delayed senescence, increased ABA sensitivity, increased yield, increased productivity and increased biomass compared to a wild type plant.

```
        10          20          30          40          50          60
ATGGAGATTC AGCGAGATAA GCAATTGGAT TATCTGATGA AAGGCTTAAG GCAGCTTGGT
        70          80          90         100         110         120
CCGCAGTTTT CTTCCTTAGA TGCTAAGTAA GTGACATGAT GCTTGGCTTC TTGTTTTCAT
       130         140         150         160         170         180
GAATTTCTTA GTACATTTTG TCCAGTGAGA GAGTAAAGCT TTGGAGCTTT GCCAATAGAC
       190         200         210         220         230         240
TTAGAAGTTT GATTTTGGCT TTTTGGATTT TGGAACAGTC GACCTTGGCT TTGTTACTGG
       250         260         270         280         290         300
ATTCTTCATT CAATAGCTTT GCTTGGGGAG ACTGTGGATG ATGAATTAGA AAGCAATGCC
       310         320         330         340         350         360
ATTGACTTCC TTGGACGCTG CCAGGTTAGT CTCAATTCCT TTTGCTTGTA CCCAATCATG
       370         380         390         400         410         420
AAAACTCTTC ATATTTGCTC TTGCATTCTT CTTGATTTTC TGCTCCTTTA GTTCACGTTT
       430         440         450         460         470         480
TCTTTTCCCG TTGCTATTAG TGTTATCTGT TATTGTTCTT TATGTACTTA GTTTGCTTTC
       490         500         510         520         530         540
TCATGTCGCT TGTCAGGGCT CTGAAGGTGG ATACGGTGGT GGTCCTGGCC AAGTAAGTAT
       550         560         570         580         590         600
ATGTCTGTTT CTTTAAAGTG TGTGGATCAC TTTCATTTCA TGCAATTGGA GAATAAACAT
       610         620         630         640         650         660
TGAGACCAGA TTATTTTATT CTGCCAGATC TCTTTTAGGT GTTTTTTTTA TGCATCATCT
       670         680         690         700         710         720
CATTGTTTGG TTGTGATGCC TTTAATTCAA GCAGCACACG TAGTTTAAGT TTAAGTTTTT
       730         740         750         760         770         780
TTCTGTGAAG ACGTAAAATG GTGTCTTTAG TTCAAGCAGC ATTTAGTTGT TTAAGTTTGT
       790         800         810         820         830         840
GGTTGTAAAT TTTCCAAACA TGGCAGAGAA AGTTAGGATA TATAACTTTT GGTCTGCCTT
       850         860         870         880         890         900
TTTCAGTTTC CTTTTTTTTT CTACTAGTAA TGGAGATATT TTTTCCCAGC TTCCACATCT
       910         920         930         940         950         960
TGCAACTACT TATGCTGCAG TGAATGCACT TGTTACTTTA GGAGGTGACA AAGCCCTTTC
       970         980         990        1000        1010        1020
TTCAATTAAT AGGTGGTGCA TTCTTTTTTC TTTGTGGTCA GTTTCTTTTA TTAAGAGTCT
      1030        1040        1050        1060        1070        1080
AGTGATGTTT CCTCTAGAAT ACTTACATGT GACTCATTCT TCTTTCAGAG AAAAAATGTC
      1090        1100        1110        1120        1130        1140
TTGTTTTTTA AGACGGATGA AGGATACAAG TGGAGGTTTC AGGTTTGATT CTCTTTCTGC
      1150        1160        1170        1180        1190        1200
TTGAACTTCT TAAAGGCATC ATTTTTACTG ACAGCGCACT CTTTATGCAT TCGTATCGCT
      1210        1220        1230        1240        1250        1260
GTTAATGCCA TACCTTCAGT CATGTTGTTT TTTTAATTCT TGCTTAATTC TACTTACTCA
      1270        1280        1290        1300        1310        1320
CTGATCGTTA GGATGCATGA TATGGGAGAA ATTGATGTTC GTGCATGCTA CACTGCAATT
```

FIGURE 1A

```
      1330        1340        1350        1360        1370        1380
TCGGTGAGTT TTACCAACTT CTATTTTCCT TTTCTCTGTT TTTGTGGACA CCAAAACTTT
      1390        1400        1410        1420        1430        1440
TTAGGATTAA TGAGATCAAC AAAGTCTGGA CCCATTATGC TATGTTTCTT CCGTTTTCAT
      1450        1460        1470        1480        1490        1500
GGCTTAAACA TCACATTCAG ATTACGATAT GATCTTATTA TTTGCACACT TGCGCCCACC
      1510        1520        1530        1540        1550        1560
AGGATACTTT GAATAGAGAT TACTCGTTTT GAGACTTACA CGTCTTGCAA ATGCATCCTA
      1570        1580        1590        1600        1610        1620
TGGCTGGTTT TCTCCCTGAT ATGTTTGACT TCTCTCTTGT GACACAGGTT GCAAGCATCC
      1630        1640        1650        1660        1670        1680
TAAATATTAT GGATGATGAA CTCACCCAGG GCCTAGGAGA TTACATCTTG AGGTAGCTTT
      1690        1700        1710        1720        1730        1740
TCTTATTACT TTTATCTCGC ATTATATATA TATAGCTGAA CTACTGTTAT ACAGTTGTAA
      1750        1760        1770        1780        1790        1800
ATTCAGGAAT TCATTAATTT CCCTGGGAAA GCTCTTTTAA CTCGATTTAT ATTGAGCAGT
      1810        1820        1830        1840        1850        1860
TGCCAAACTT ATGAAGGTGG CATTGGAGGG GAACCTGGCT CCGAAGCTCA CGGTGGGTAT
      1870        1880        1890        1900        1910        1920
GGTCTCCAAC TAACTTCCAT TATGTTGAGG CTTAGATAAA AATTGTGCTT TGCTTCCCTC
      1930        1940        1950        1960        1970        1980
TTCCTTGATG ACATGGTTAT TGATGGTTAA GTATAATTAA TTTTCTGAAA TAGGATTTGT
      1990        2000        2010        2020        2030        2040
CACCTGCAGC TTGCATGCCT GCCGCTTTGC TTATTACCAA GTTGTTTTTT GTTTAGGTAT
      2050        2060        2070        2080        2090        2100
ACCTACTGTG GTTTGGCTGC TATGATTTTA ATCAATGAGG TCGACCCGTT TGAATTTGGA
      2110        2120        2130        2140        2150        2160
TTCATTAATG GTAACATACA ATGCTGTTTG GAGATGATTA ATAATTTTCC CTGAGAGATA
      2170        2180        2190        2200        2210        2220
TTTTCCTTAC CAAATAATTT CCTTATGATT CTAGAATTGG GCTGTACATC GACAAGGAGT
      2230        2240        2250        2260        2270        2280
AGAAATGGGA TTTCAAGGTA GGACGAACAA ATTGGTCGAT GGTTGCTACA CATTTTGGCA
      2290        2300        2310        2320        2330        2340
GGTTAACTTT CTATCTTTCA GGATTATTAT TGGCCCTACT TCTAAATTCT TCACCGTTGT
      2350        2360        2370        2380        2390        2400
TGTCTTTTCT TATTTCCTTT GGGTATATGT TAAACAGGCA GCCCCTTGTG TTCTACTACA
      2410        2420        2430        2440        2450        2460
AAGATTATAT TCAACCAATG ATCATGACGT TCATGGATCA TCACATATAT CAGAAGGGAC
      2470        2480        2490        2500        2510        2520
AAATGAAGAA CATCATGCTC ATGATGAAGA TGACCTTGAA GACAGTGATG ATGATGATGA
      2530        2540        2550        2560        2570        2580
TTCTGATGAG GACAACGATG AAGGTATTCA ATCAAATTTC TCAACCATCA AGTCCATCTG
      2590        2600        2610        2620        2630        2640
ATAATTCAAA ACACAACGAA ATTTTAGTTA GCTTATATTT GCAGATTCAG TGAATGGTCA
```

FIGURE 1B

```
      2650        2660        2670        2680        2690        2700
CAGAATCCAT CATACATCCA CCTACATTAA CAGGAGAATG CAACTGGTTT TTGATAGCCT
      2710        2720        2730        2740        2750        2760
CGG?TTGCAG AGATATGTAC TCTTGTGCTC TAAGGTCAGT CCAGAACAAA ACATCCAGTC
      2770        2780        2790        2800        2810        2820
AAGTTAACAC TTAACATTTG TATAACACAA GCACACACAC TTGTATGCGC AGATCCCTGA
      2830        2840        2850        2860        2870        2880
CGGTGGATTC AGAGACAAGC CGAGGAAACC CCGTGACTTC TACCACACAT GTTACTGCCT
      2890        2900        2910        2920        2930        2940
GAGCGGCTTG TCTGTGGCTC AGCACGCTTG GTTAAAAGAC GAGGACACTC CTCCTTTGAC
      2950        2960        2970        2980        2990        3000
TCGCGACATT ATGGGTGGCT ACTCGAATCT CCTTGAACCT GTTCAACTTC TTCACAACAT
      3010        3020        3030        3040        3050        3060
TGTCATGGAT CAGTATAATG AAGCTATCGA GTTCTTCTTT AAAGCAGCAT GACCCGTTGT
      3070        3080        3090        3100        3110        3120
TGCTAATGTA TGGGAAACCC CAAACATAAG AGTTTCCGTA GTGTTGTAAC TTGTAAGATT
      3130        3140        3150        3160        3170        3180
TCAAAAGAAG TTTCACTAAT TTAACCTTAA AACCTGTTAC TTTTTATTAC GTATA.....
```

FIGURE 1C

MEIQRDKQLDYLMKGLRQLGPQFSSLDANRPWLCYWILHSIAL
LGETVDDELESNAIDFLGRCQGSEGGYGGGPGQLPHLA
TTYAAVNALVTLGGDKALSSINREKMSCFLRRMKDTSGGFR
MHDMGEIDVRACYTAISVASILNIMDDELTQGLGDYILS
CQTYEGGIGGEPGSEAHGGYTYCGLAAMILINEVDRLNLDSL
MNWAVHRQGVEMGFQGRTNKLVDGCYTFWQAAPCVLLQ
RLYSTNDHDVHGSSHISEGTNEEHHAHDEDDLEDSDDDDSDE
DNDEDSVNGHRIHHTSTYINRRMQLVFDSLGLQRYVL
LCSEIPDGGFRDKPRKPRDFYHACYCLSGLSVAQHAWLKDED
TPPLTRDIMGGYSNLLEPVQLLHNIVMDQYNEAIEFFF
KAA

FIGURE 2

```
        10          20          30          40          50          60
CTCACTCATT AGCACCCCAG CTTTACACTT TATGCTTCCG CTCGTATGTT GTGTGGAATT
        70          80          90         100         110         120
GTGAGCGATA ACAATTTC?A CACAGGAAAC AGCTATGACA TGATTACGAA TTCAAAAAAA
       130         140         150         160         170         180
TAGAGATTGG CAATATTTTA GTGTGTGAAT AATATTCATC CCTAAAAAGA AGTCATCTTT
       190         200         210         220         230         240
CGACTTTGTG GCAACAGTTC TGTTATTAAA ATGTGTGAGC GTGACATATT TTGAAGAGGT
       250         260         270         280         290         300
ACCTCGACAA AATCGGAAGG TGTCTCATTT TCTTCTATCG GAAGGCTTTC TCGTTGAAGG
       310         320         330         340         350         360
TAGTCGTTGT AGCTGAAAAA TTAAGAAAAC CTAGTGAGCT CTTCATGTAT TCAAAAATTC
       370         380         390         400         410         420
AACCAGTGTA ATCAAACTCA AGAGGTAAAT AGTTAAAATC CCATACCAAA CCGTGTAATC
       430         440         450         460         470         480
TATGCAATAC CTAATTAACA AAGTTAAAAG CGTTAGTCTA GCAGTAATAT TGTATCAAAA
       490         500         510         520         530         540
GCTCTAACAG TAATTAATAA CCAGTGTCAC CAGAAACAAA TGTCAATAAC ATGGAAAATT
       550         560         570         580         590         600
GAATTTAGTT GAGTCCTGGA GGTCGTGGAC GTCGTGGAGG CTGTGGACGT CGTGAATACG
       610         620         630         640         650         660
CATAAAGAAA AATCTTATAA TCGTGCAAAT ATTCACCGTT CTTCTTATAC ATCACCTACG
       670         680         690         700         710         720
GTAATAAAAG AGTTTTATTT CAGCAATCGT ACATTCAAAT TGAAACTTAG ATACACTATA
       730         740         750         760         770         780
TATTTTTCAT CATAACTAAC TATAAACTAG TCTAAACCTT TTTTGCTTCG TTAGCAGAAG
       790         800         810         820         830         840
CAAAGTCAAC AGGCCATAGC ACCTATGGAT ACGCTTGGCG GTTACAAAAA GTCGAACACG
       850         860         870         880         890         900
AACAACTTCT CCAGCATCTT TGAAGAAATT GATGCTGTAA CAAACAGTGT AAGGTAAAAA
       910         920         930         940         950         960
TATCAGTCAT GCTCAGAGAA GGAAAGTGGA GATTGAAGAT GGTGCTACTT ACATATCTGA
       970         980         990        1000        1010        1020
TATTTTAGTT TGGGGAGGGA TATGGCCATT AAAGA?CGTC TTTTTTGTCA CCTGGATTTA
      1030        1040        1050        1060        1070        1080
ACAGCCAAGT GTGTTAGCAC AAGATTCTTA ATTGAACAGA AATTTGTACA AAATATCTAG
      1090        1100        1110        1120        1130        1140
CAAATCCGTT GGTTGTTTCC TCCTGTTACA TATGATACAA GATCAAAGAG TAGCCATTAG
      1150        1160        1170        1180        1190        1200
AAGAAGACAG TG?AAAGAAG ATTGTTTTGT CAAAGAAGAA GAGTAATACG AGGCCATCTT
      1210        1220        1230        1240        1250        1260
AGGGTTACCT TATTCTACTT ATGTCTCTTG AGAATGGAAT TGGTCACCAA ATCATCTTCT
      1270        1280        1290        1300        1310        1320
TCAGGGTTAC GCTTACCTAA AAGAAGAGCA ACAA??AAAA AACTCTTGAG ACAAGTTTAA
```

FIGURE 3A

```
      1330        1340        1350        1360        1370        1380
CACATTAGAT AAAAGAGAGA GAGAGAGAGG CAACCAAAAA CAAACCCAAT AAATTGCTAC
      1390        1400        1410        1420        1430        1440
TAGAAGTGGC CATGGAGAAG ATGAAACGAG GTTTATGTAT TTTTCCGTTA AGAGCAAGCA
      1450        1460        1470        1480        1490        1500
ATAATATAGC CCTAAAGAAA TATAGACCTA GCCTAGGAAG AAGTTTCTAA GACCATCCTT
      1510        1520        1530        1540        1550        1560
ATCAATGAAC TCTTACATAA AGTTCTAAAC AATTTTGATA TACAAAATAA TGTTTAAACA
      1570        1580        1590        1600        1610        1620
TTAGAATGGC TCTTACAAAA AAAGAGAATA AAGAAAAAAA AAACTTAGCT AAGAGCCATT
      1630        1640        1650        1660        1670        1680
TTTCATTTCT TAAGCACACT TTTTTATTTT TTTATTCTTA TTTTATTTAA TATAATATTT
      1690        1700        1710        1720        1730        1740
TGATAGTTCT TATGATATTG TTAACAACCT ATTGATAAGG ATGCTCTAAC TAATCTTATA
      1750        1760        1770        1780        1790        1800
AATAAAACAA TGAATCTGGT TTGGTCTGGG CGTAACAG?A ATTATACTCT TTTTTTTTTT
      1810        1820        1830        1840        1850        1860
TGTCAAGAGG AAATTATACT AAGAAGCAAC AGATTAAACA TTAAAGCGTA TAGTAAAATT
      1870        1880        1890        1900        1910        1920
AATTGTTTGA GAATCTTAAA CCAAACCGAA CCGGTATTAA ACCGGAACCA AATTGGCAAT
      1930        1940        1950        1960        1970        1980
GAAATTTAGA TGCCAGTAGT AACCCGCTTG ATTCGTTTGA AGTGTGTAGG GCTCAGACTT
      1990        2000        2010        2020        2030        2040
GACCGGAGTG GACTCAATCG GCGAATCTGT CACGGAGGAC ACGGGGAATC AACGCGGCGG
      2050        2060        2070        2080        2090        2100
AGAGTGATGG AAGAGTTTTC AAGCCTAACC GTGAGTCAGC GCGAGCAATT TCTGGTGGAG
      2110        21200       2130        2140        2150        2160
AACGATGTGT TCGGGATCTA TAATTACTTC GACGCCAGCG ACGTTTCTAC TCAAAAATAC
      2170        2180        2190        2200        2210        2220
ATGTAAGCTG ACGGATTGAT TTTCTAGTTT TCTTCATGAT CTGATGAATT TTAGTAGCGT
      2230        2240        2250        2260        2270        2280
CGTGAAAGAA TTATTTTCGT CGATAGATGA ATCTTACTGA TATGGAAGTT GTTCTATCCT
      2290        2300        2310        2320        2330        2340
AGGATG ... .......... .......... .......... .......... ..........
   └-First Codon
```

FIGURE 3B

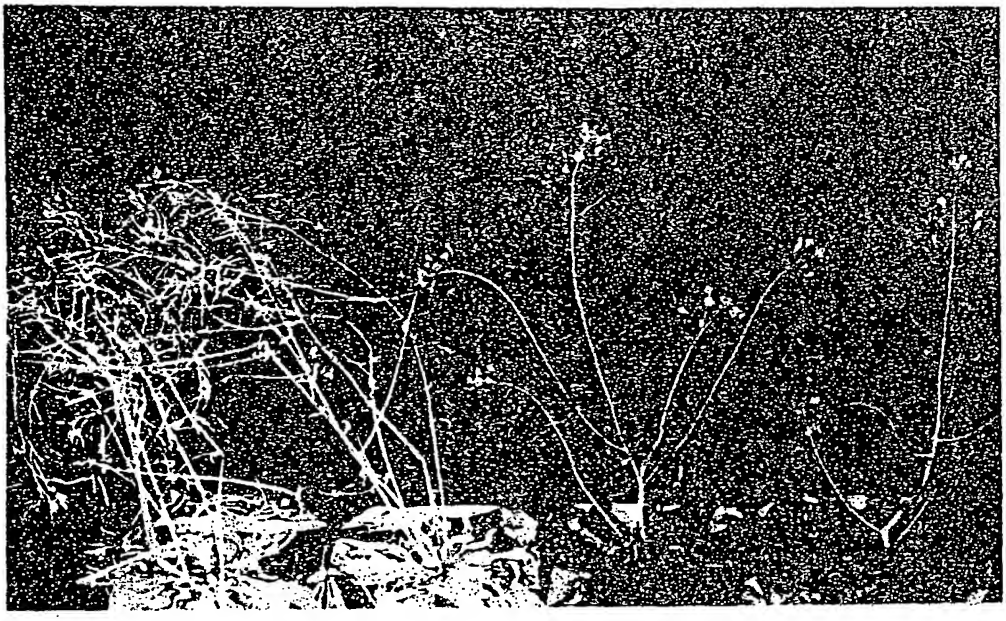
```
                                                                      29
Arab.       MEIQRDKQLD YLMKGLRQLG PQFSSLDAN- ---------- ----------
Pea         ..ASTAAETP TPTVSQ.DQW IVE.QVFHIY QLFANIPPNA QSII------
Yeast       .RQRVGRSIA RAKFINTA.L GRKRPVMERV VDIAHVDSSK AIQPLMKELE
Rat         .ASSSSFTYY CPPSSSPVWS EPLY..RPEH ARERLQDDSV ETVTSIEQAK

Arab.       ---------- ---------- ---------- ---------- ----------
Pea         ---------- ---------- ---------- ---------- ----------
Yeast       TDTTEARYKV LQSVLEIYDD EKNIEPALTK EFHKMYLDVA FEISLPPQMT
Rat         VEEKIQEVFS SYKFNHLVPR LVLQREKHFH YLKRGLRQ-- ----LTDAYE
                                                                      73
Arab.       -----RPWLC YWILHSIALL G-ETVDDELE SNAIDFLGRC QGSEGGYGGG
Pea         ----------. ...I...... .-.SI..D.. D.TV...N.. .DPN...A..
Yeast       ALDASQ..ML ...AN.LKVM DRDWLS.DTK RKIV.K.FTI SP.G.PF...
Rat         CLDAS..... ......LE.. D-.PIPQIVA TDVCQ..EL. .SPD..F...
                                                                     122
Arab.       PGQLPHLATT YAAVNALVTL GGDKALSS-I NREKMSCFLR RMKDTSGG.R
Pea         ...M...... .....T.I.. ..E.S.A.-. ..N.LYG.M. ...QPN....
Yeast       ....S...S. ...I...SLC DNIDGCWDR. D.KGIYQW.I SL.EPN...K
Rat         ...Y....P. .......CII .TEE.YNV-. ....LLQY.Y SL.QPD.S.L
                                                                     171
Arab.       -MHDMGEIDV RACYTAISVA SILNIMDDEL TQGLGDYILS CQTYEGGIGG
Pea         -...E..... .......... .V...L.... IKNV..F... .......LA.
Yeast       TCLEV..V.T .GI.C.L.I. TL...LTE.. .E.VLN.LKN ..N....F.S
Rat         -..VG..V.. .SA.C.A... .LT..ITPD. FE.TAEW.AR ..NW......
                                                                     218
Arab.       EP-GSEAHGG YTYCGL.AM- ILINEVDRLN LDSLMNWAVH RQGV-EMGFQ
Pea         ..-........ ..F......- ...G..N..D .PR.LD.V.F ...K-.C...
Yeast       C.HVD..... ..F.AT.SLA ..RSM-.QI. VEK.LE.SSA ..LQE.R..C
Rat         V.-.M..... ..F.....LV ..KK.-RS.. .K..LQ.VTS ..MRF.G...
                                                                     267
Arab.       GRTNKLVDGC YTFWQAAPCV LLQR-LYSTN DHDVHGSSHI SEGTNEEHHA
Pea         .......... .S...GGAVA ....-.H.II .EQMAEA.QF VTVSDAPEEK
Yeast       ..S........ .S..VGGSAA I.EAFG.GQC ---------- ----------
Rat         ..C........ .S....GLLP ..H.A.HAQG .PALSM.--- ----------
                                                                     316
Arab.       HDEDDLEDSD DDDDSDEDND EDSVNGHRIH HTSTYINRRM -QLVFDSLGL
Pea         ECL.GTSSHA TSHIRH.GMN .SCSSDVKNI GYNFISEW.Q SEPL.H.IA.
Yeast       ---------- ---------- ---------- ---------- -----.NKHA.
Rat         ---------- ---------- ---------- ---------- -HWM.HQQA.
                                                                     364
Arab.       QRYVLLCSKI -PDGGFRDKP RKPRDFYHTC YCLSGLSVAQ HAWLKDE-DT
Pea         .Q.I....QE -Q...L.... G.R..H..S. .......LC. YS.S.RP-.S
Yeast       RD.I.Y.CQE KEQP.L.... GAHS.....N ...L..A..E SSYSCTPN.S
Rat         .E.I.M.CQC -.A..LL... G.S....... .......I.. -----HFGSG
                                                                     404
Arab.       PPLTRDIMGG YSNLLEPVQL LHNIVMDQYN EAIEFFFKAA ----------
Pea         ...PKVV..P .......IHP .F.V.L.R.R ..H...SQL- ----------
Yeast       .HNIKCTPDR LIGSSKLTDV NPVYGLPIE. VRKIIHYFKS NLSSPS----
Rat         AM.HDVV..V PE.V.Q.THP VY..GP.KVI Q.TTH.LQKP VPGFEECEDA

Arab.       ----------
Pea         ----------
Yeast       ----------
Rat         VTSDPATD--
```

FIGURE 4

FIG.5



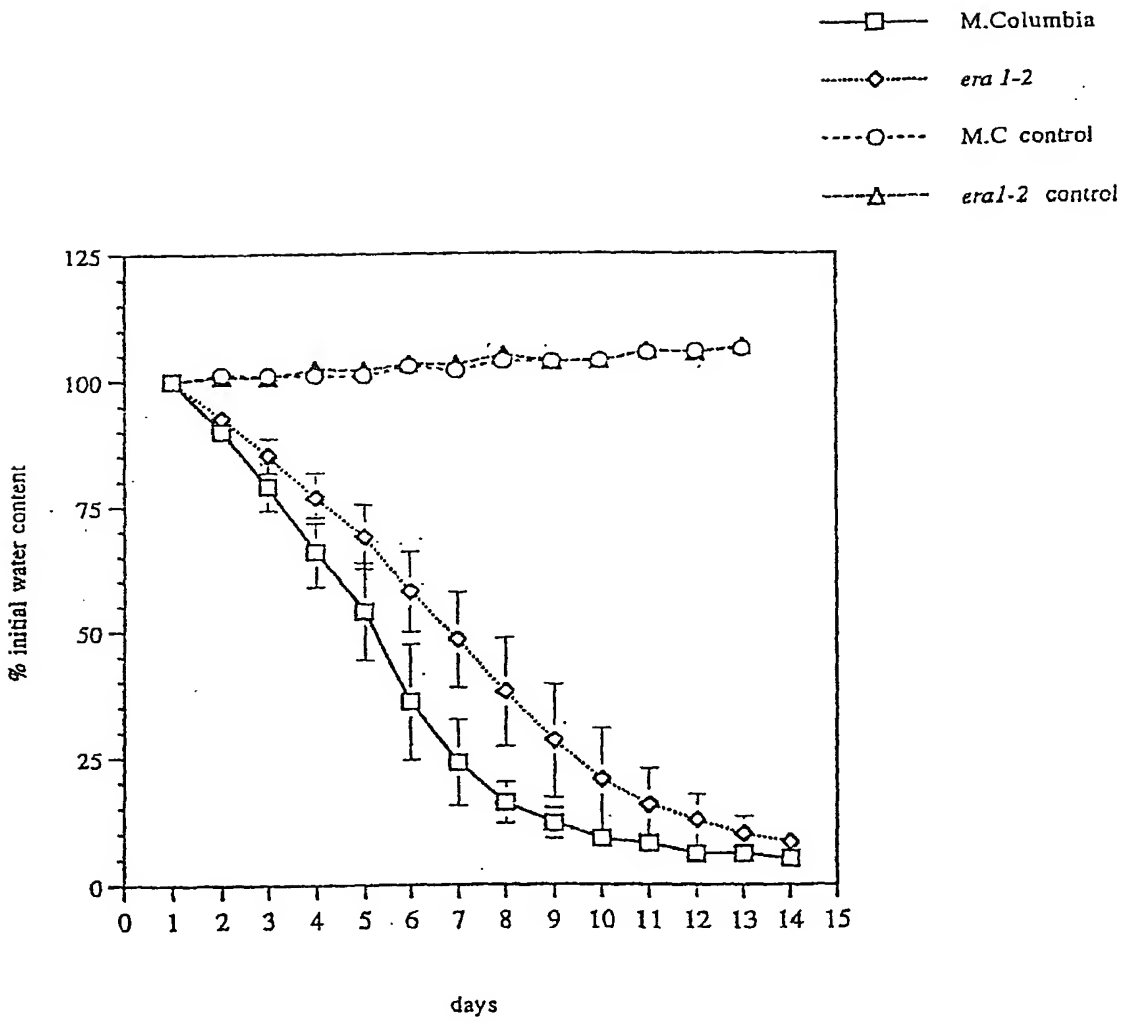

M.Columbia
era 1-2
M.C control
era1-2 control
% initial water content
125
100
75
50
25
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
days

FIGURE 6

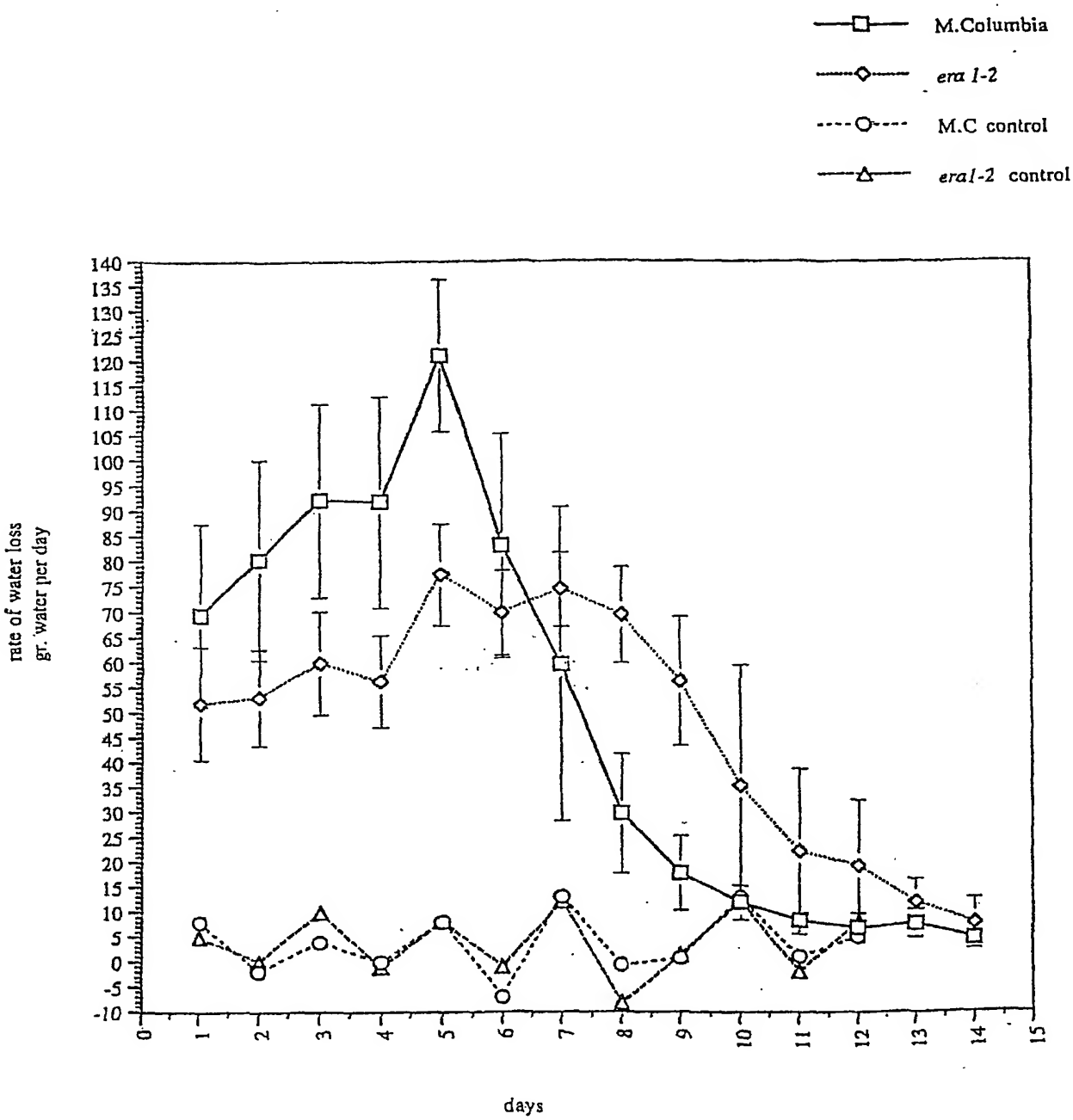

M.Columbia
era 1-2
M.C control
era1-2 control
rate of water loss
gr. water per day
days
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
-5
-10
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15

FIGURE 7

FIG.8A

Day 0

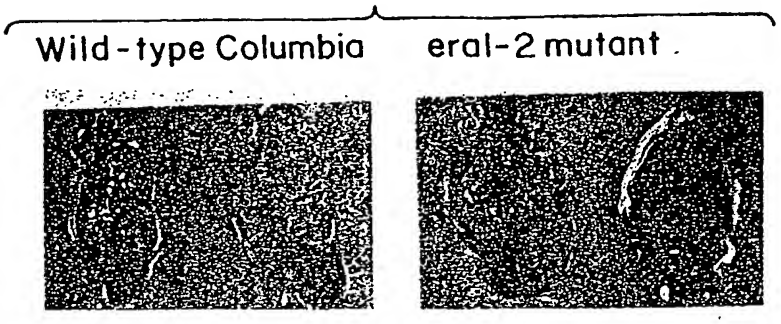


FIG.8B

Day 3

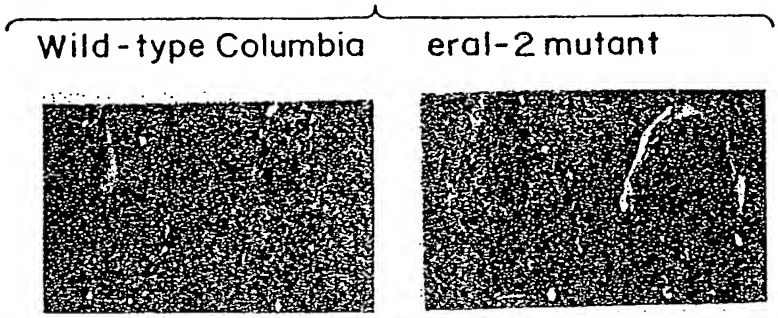


FIG.8C

Day 6

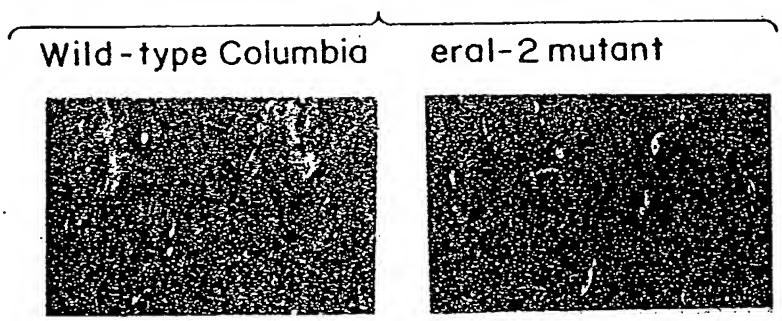


FIG.8D

Day 9

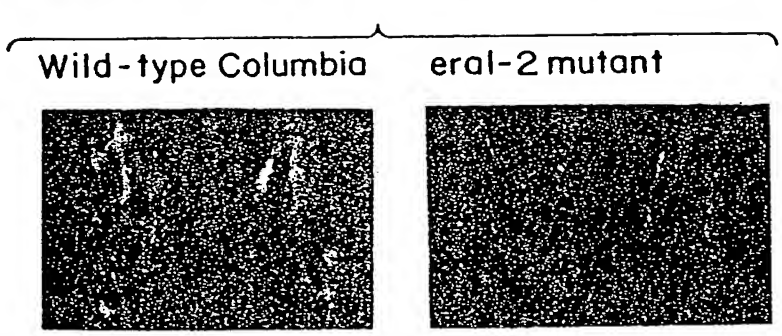
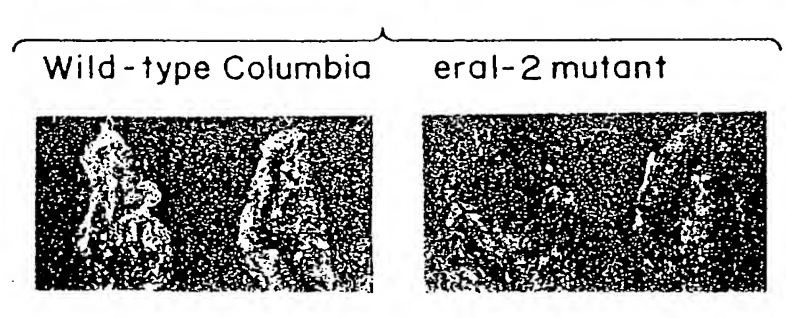


FIG.8E

Day 12



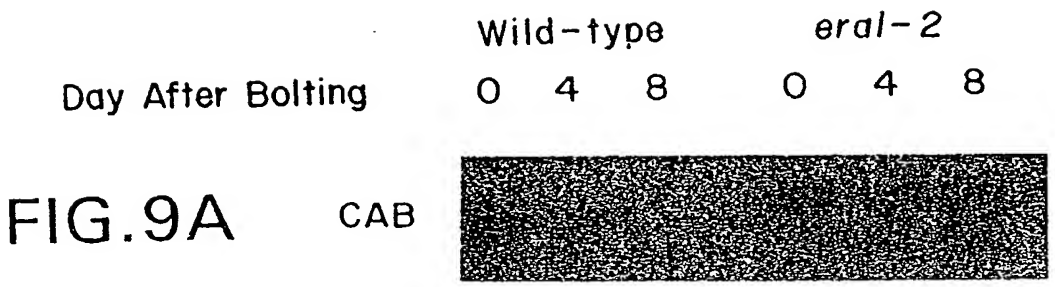


FIG.9A CAB

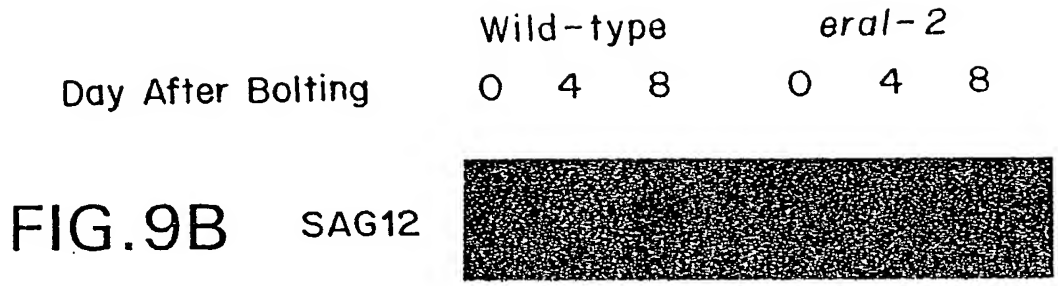


FIG.9B SAG12

FIG.9C SAG13

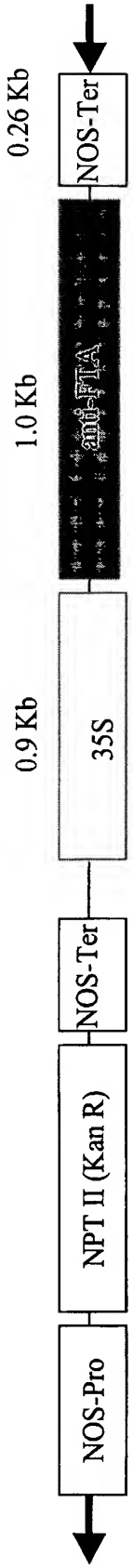

0.26 Kb
NOS-Ter
1.0 Kb
anti-FT-A
0.9 Kb
35S
NOS-Ter
NPT II (Kan R)
NOS-Pro

Figure 10

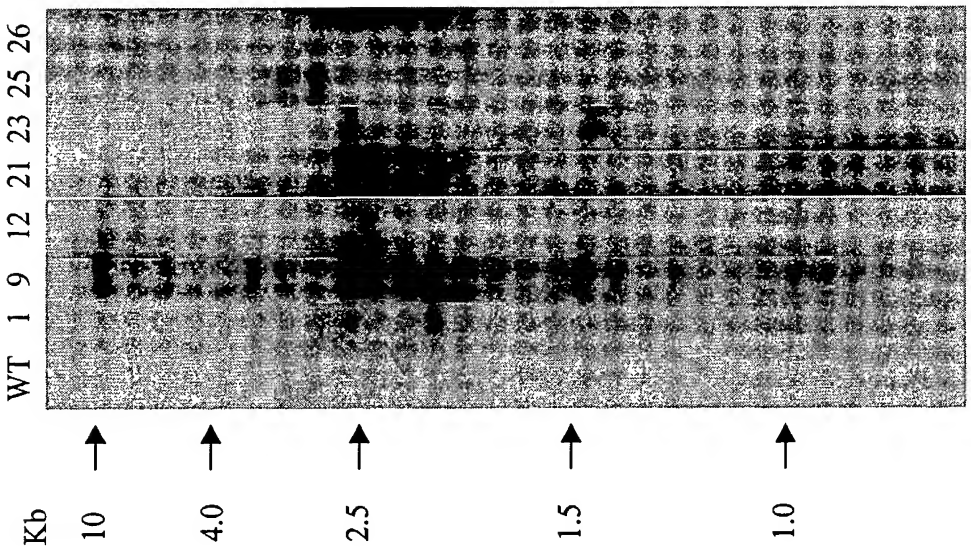


Figure 11

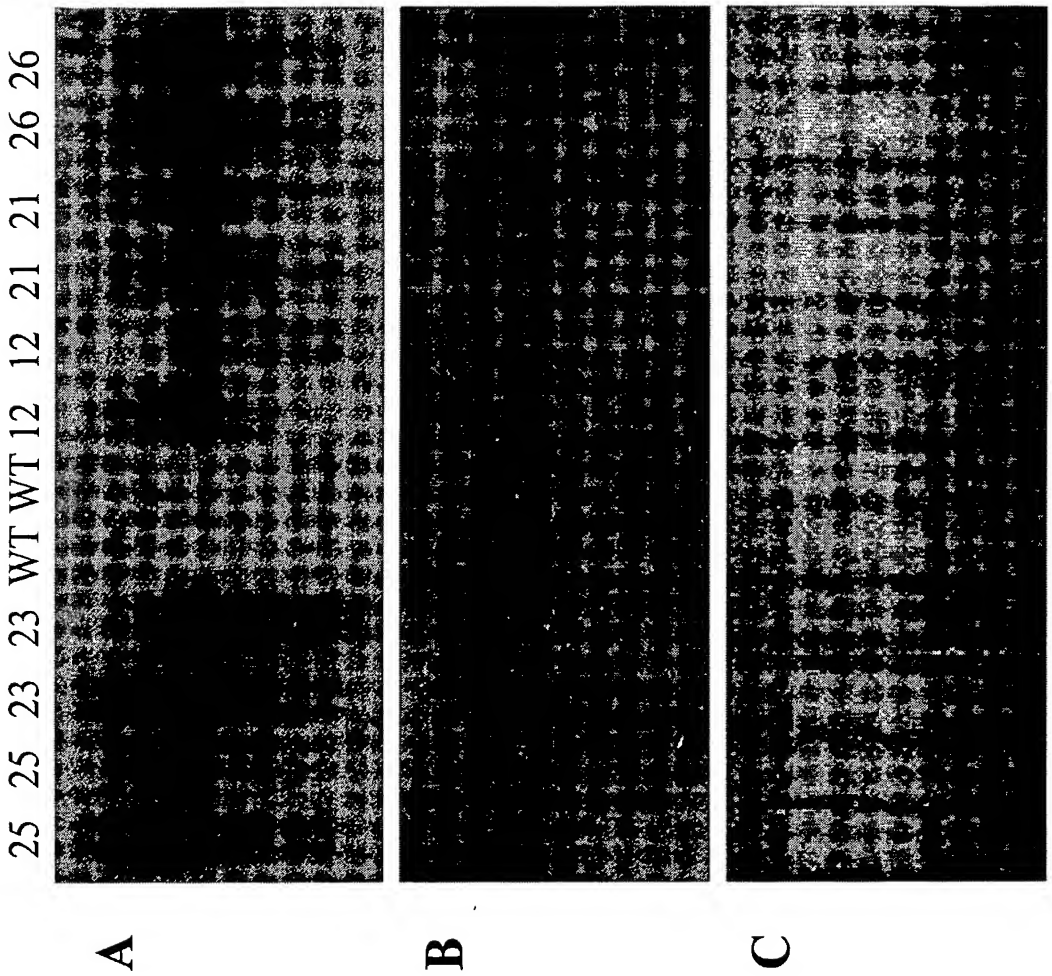


Figure 12

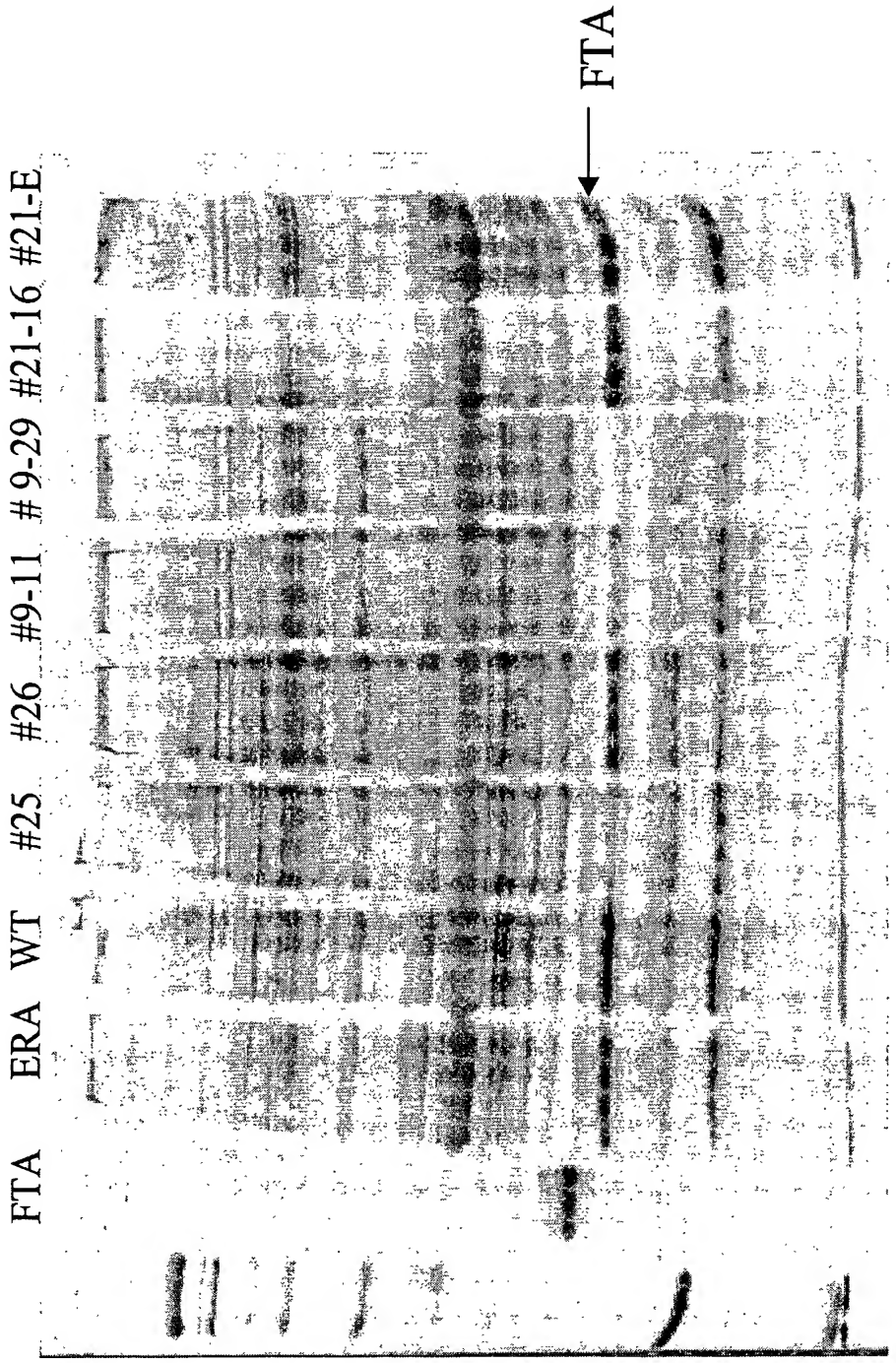


Figure 13

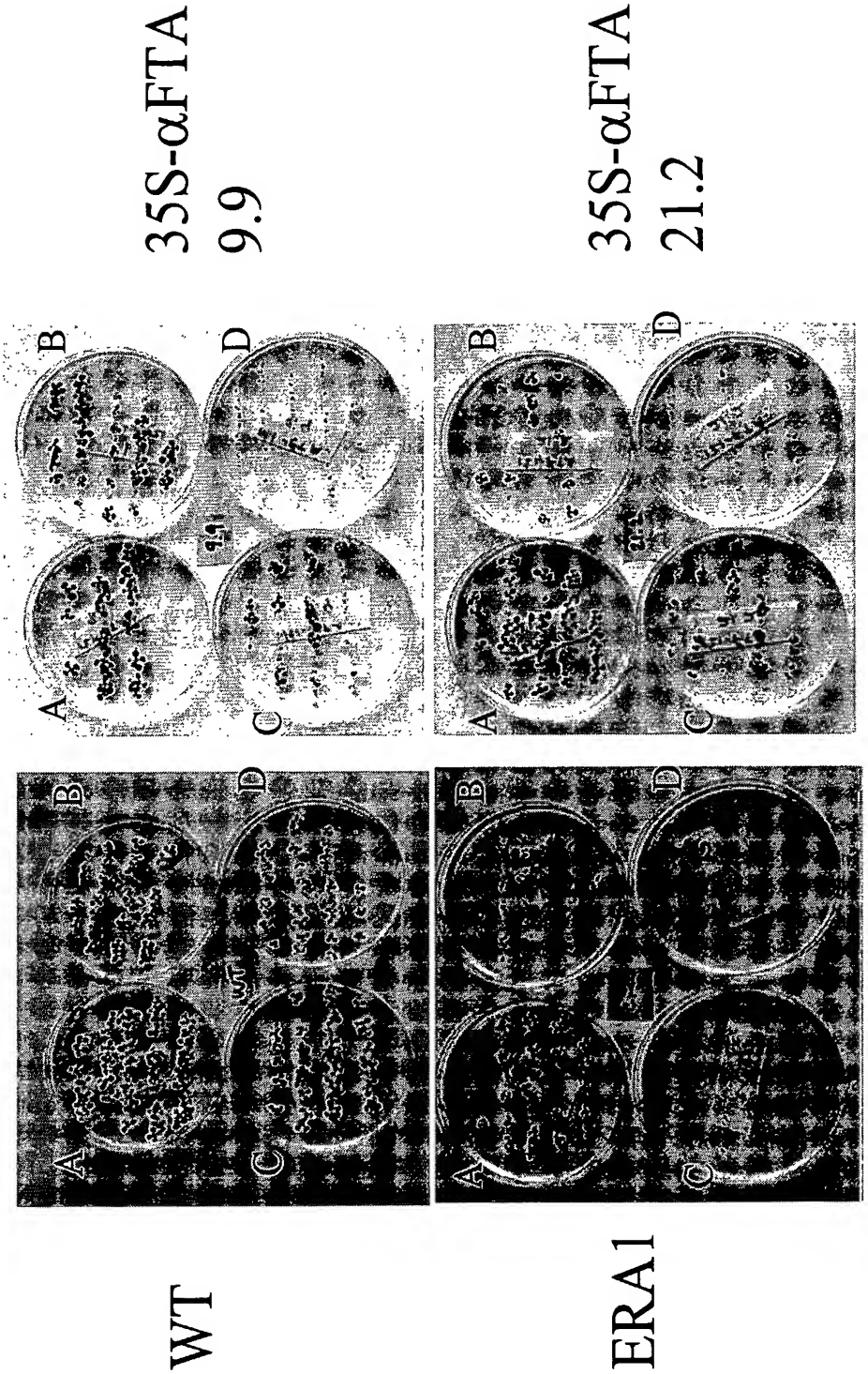


Figure 14

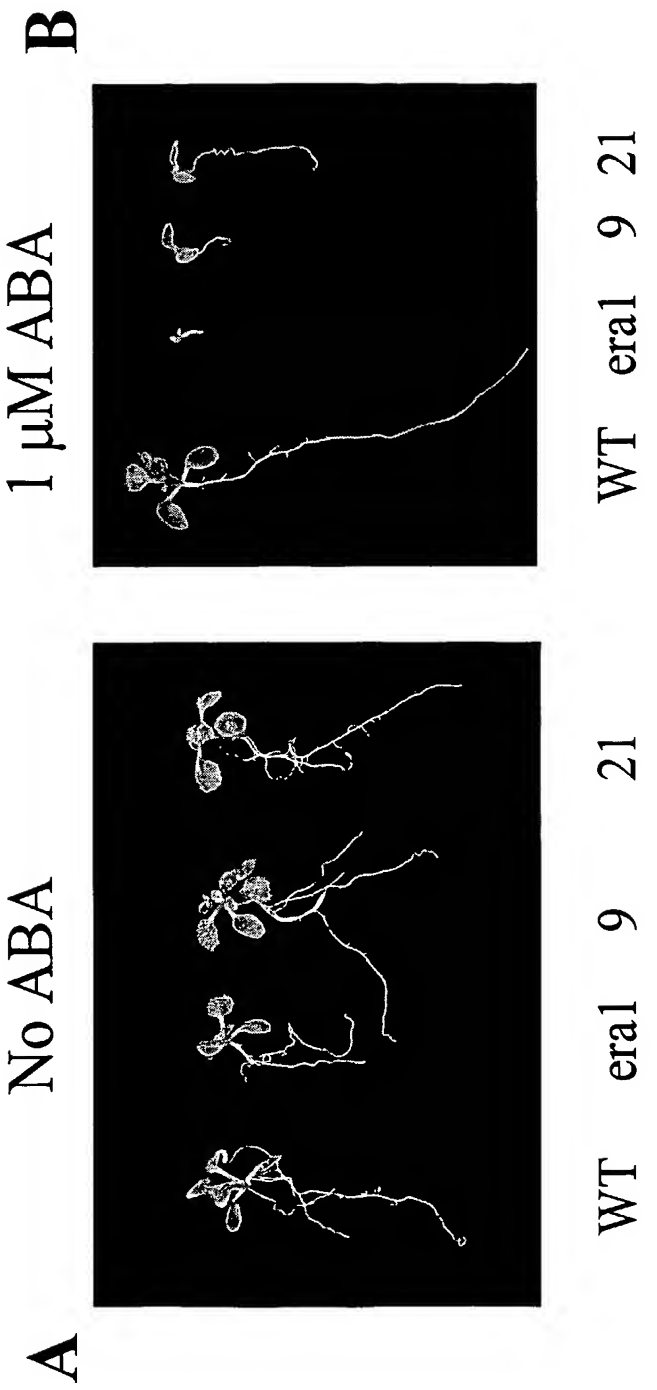


Figure 15

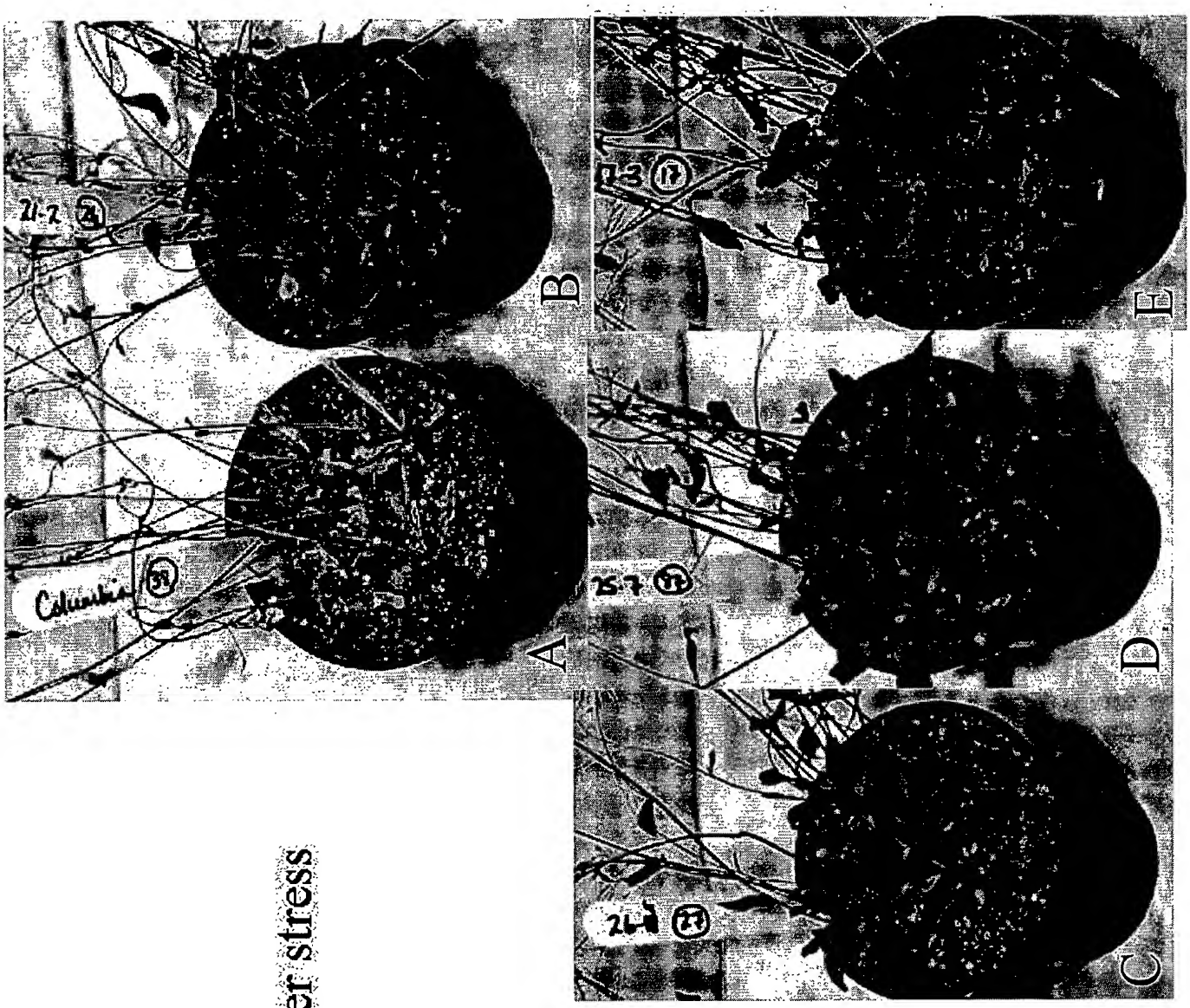


Figure 16.
Day 8 of water stress

DNA	Brassica napus	Arabidopsis thaliana	PPI Glycine max	Zea mays	Rice	Soy 1	Soy 2	Triticum	Tomato	Pea
Brassica napus	X									
Arabidopsis thaliana	89	X								
PPI Glycine max	61	55	X							
Zea mays	57	45	52	X						
Rice	55	46	54	63	X					
Soy 1	61	50	98	43	47	X				
Soy 2	61	50	99	41	46	99	X			
Triticum	58	45	52	56	66	43	41	X		
Tomato	65	53	63	44	51	52	49	41	X	
Pea	66	55	78	46	50	70	69	44	49	X
PROTEIN	Brassica napus	Arabidopsis thaliana	PPI Glycine max	Pea	Tomato	Rice	Zea mays	Soy 1	Soy 2	Triticum
Brassica napus	X									
Arabidopsis thaliana	89	X								
PPI Glycine max	65	63	X							
Pea	61	61	77	X						
Tomato	60	59	57	58	X					
Rice	64	63	56	58	58	X				
Zea mays	61	56	58	57	56	75	X			
Soy 1	66	64	98	77	58	57	58	X		
Soy 2	66	64	98	78	58	57	58	99	X	
Triticum	61	60	57	59	60	80	73	58	58	X

Figure 17

DNA	Brassica napus	Arabidopsis thaliana	Wiggum	PPI Glycine max	Glycine max	PPI Zea maize	Zea maize	Pea	Tomato	Tobacco
Brassica napus	X									
Arabidopsis thaliana	88	X								
Wiggum	88	99	X							
PPI Glycine max	60	64	65	X						
Glycine max	60	64	65	99	X					
PPI Zea maize	38	54	59	63	63	X				
Zea maize	54	54	59	62	62	99	X			
Pea	65	57	45	78	77	56	56	X		
Tomato	68	62	52	70	70	64	64	51	X	
Tobacco	68	64	60	71	71	65	65	55	83	X
PROTEIN	Brassica napus	Arabidopsis thaliana	Wiggum	PPI Glycine max	Glycine max	PPI Zea maize	Zea maize	Pea	Tomato	Tobacco
Brassica napus	X									
Arabidopsis thaliana	84	X								
Wiggum	84	99	X							
PPI Glycine max	54	58	59	X						
Glycine max	53	58	58	99	X					
PPI Zea maize	52	50	52	58	58	X				
Zea maize	51	50	52	58	58	99	X			
Pea	58	56	57	78	78	56	56	X		
Tomato	60	62	55	63	63	58	58	62	X	
Tobacco	62	63	59	64	63	58	58	64	83	X

Figure 18

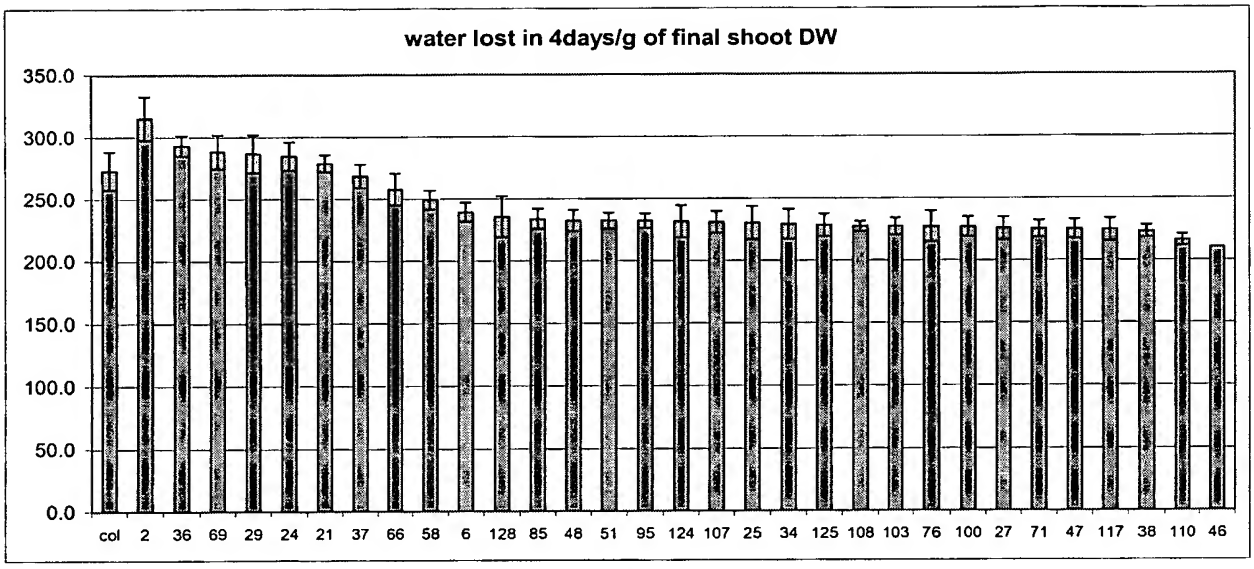

water lost in 4days/g of final shoot DW
350.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
col 2 36 69 29 24 21 37 66 58 6 128 85 48 51 95 124 107 25 34 125 108 103 76 100 27 71 47 117 38 110 46

Figure 19

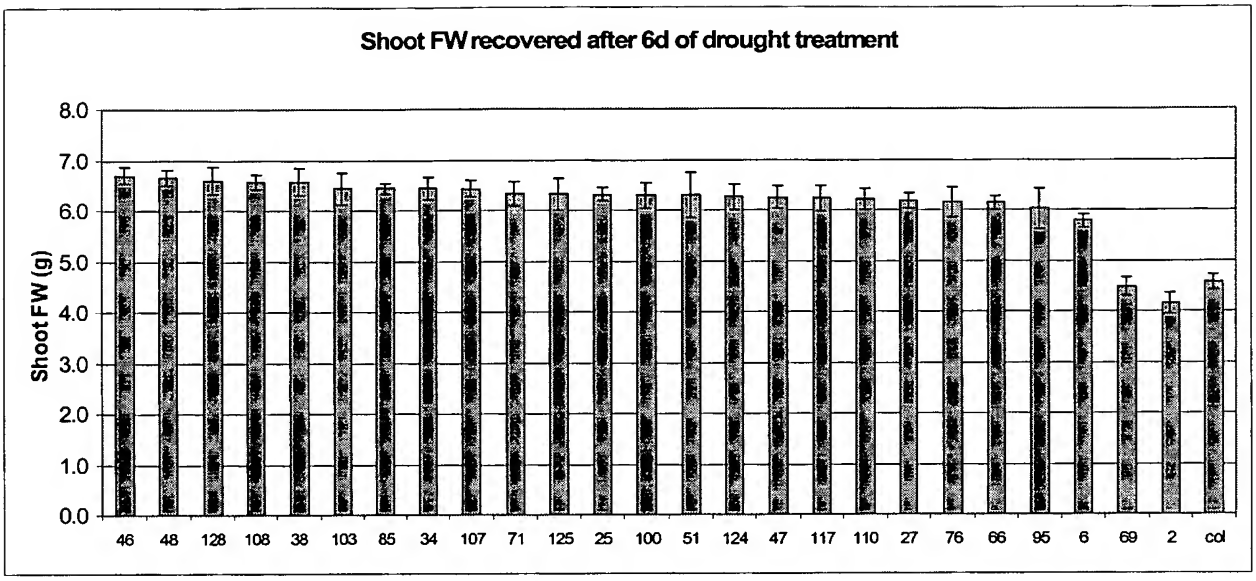

Shoot FW recovered after 6d of drought treatment
Shoot FW (g)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
46 48 128 108 38 103 85 34 107 71 125 25 100 51 124 47 117 110 27 76 66 95 6 69 2 col

Figure 20

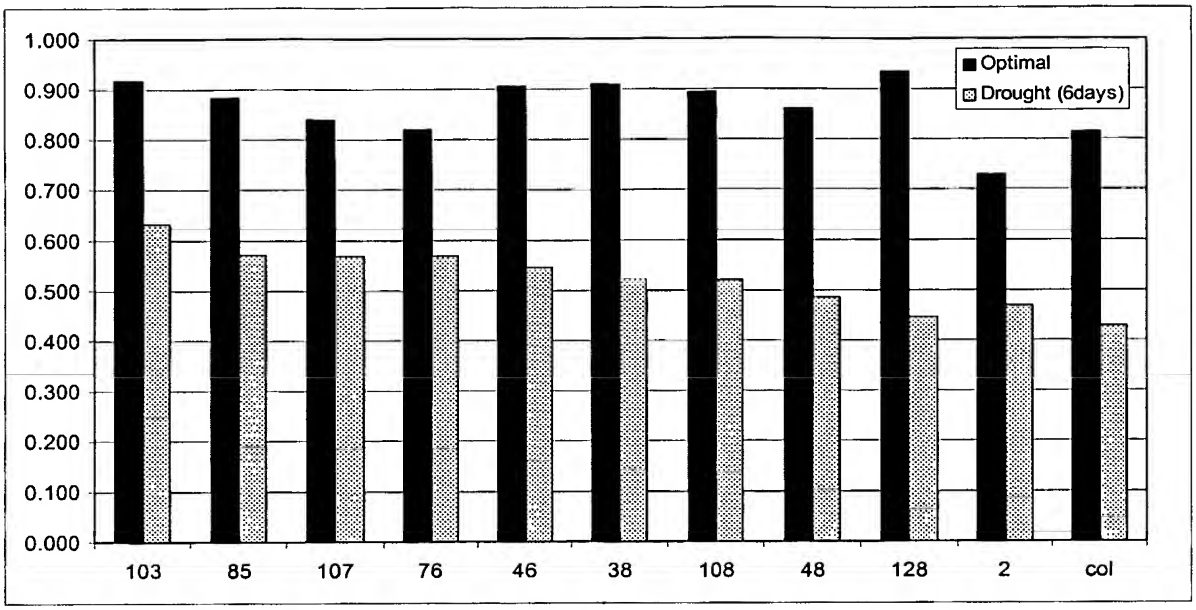

1.000
0.900
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
Optimal
Drought (6days)
103
85
107
76
46
38
108
48
128
2
col

Figure 21

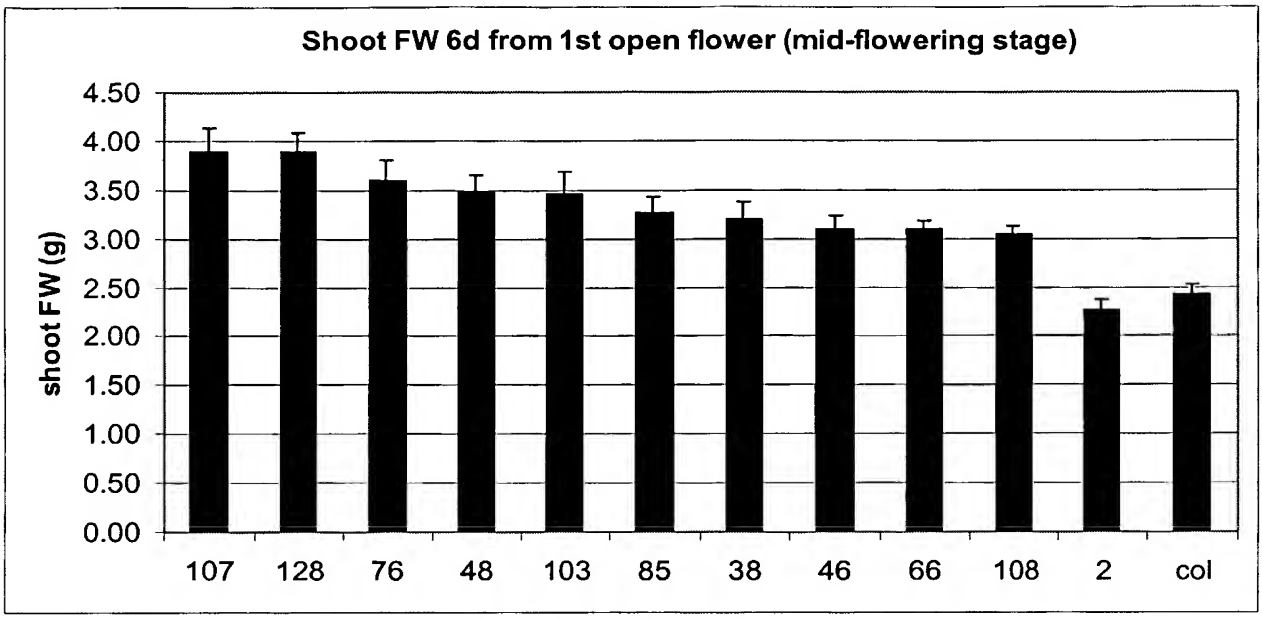

Shoot FW 6d from 1st open flower (mid-flowering stage)
shoot FW (g)
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
107
128
76
48
103
85
38
46
66
108
2
col

Figure 22

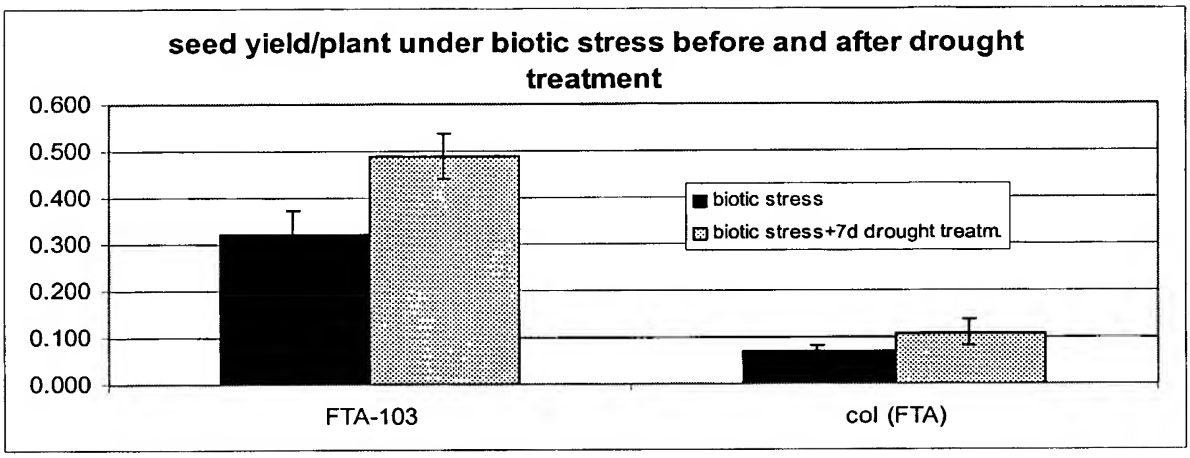

seed yield/plant under biotic stress before and after drought treatment
0.600
0.500
0.400
0.300
0.200
0.100
0.000
biotic stress
biotic stress+7d drought treatm.
FTA-103
col (FTA)

Figure 23

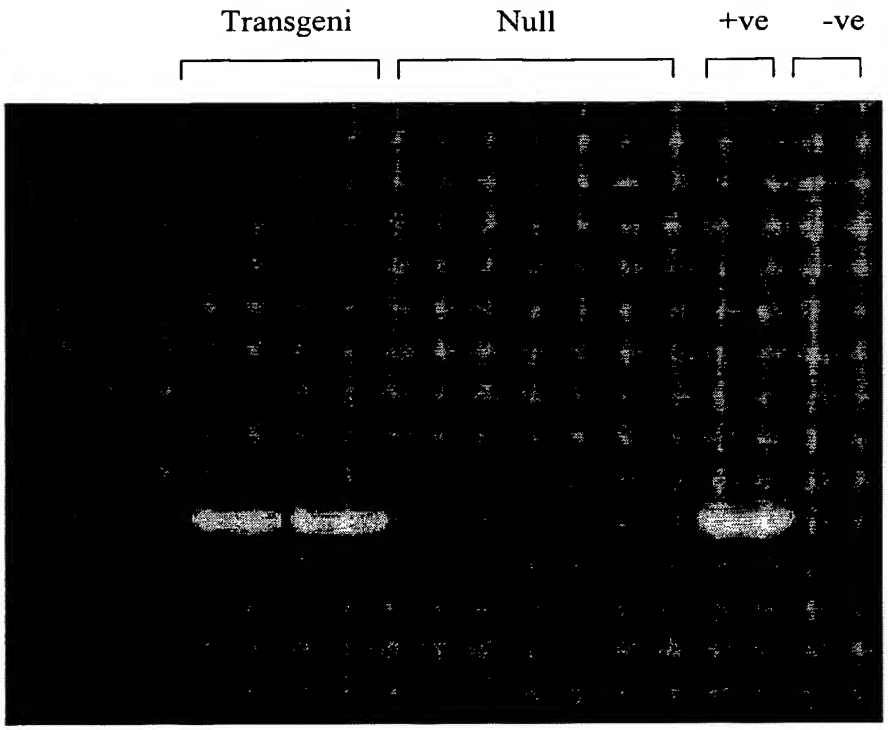


Figure 24

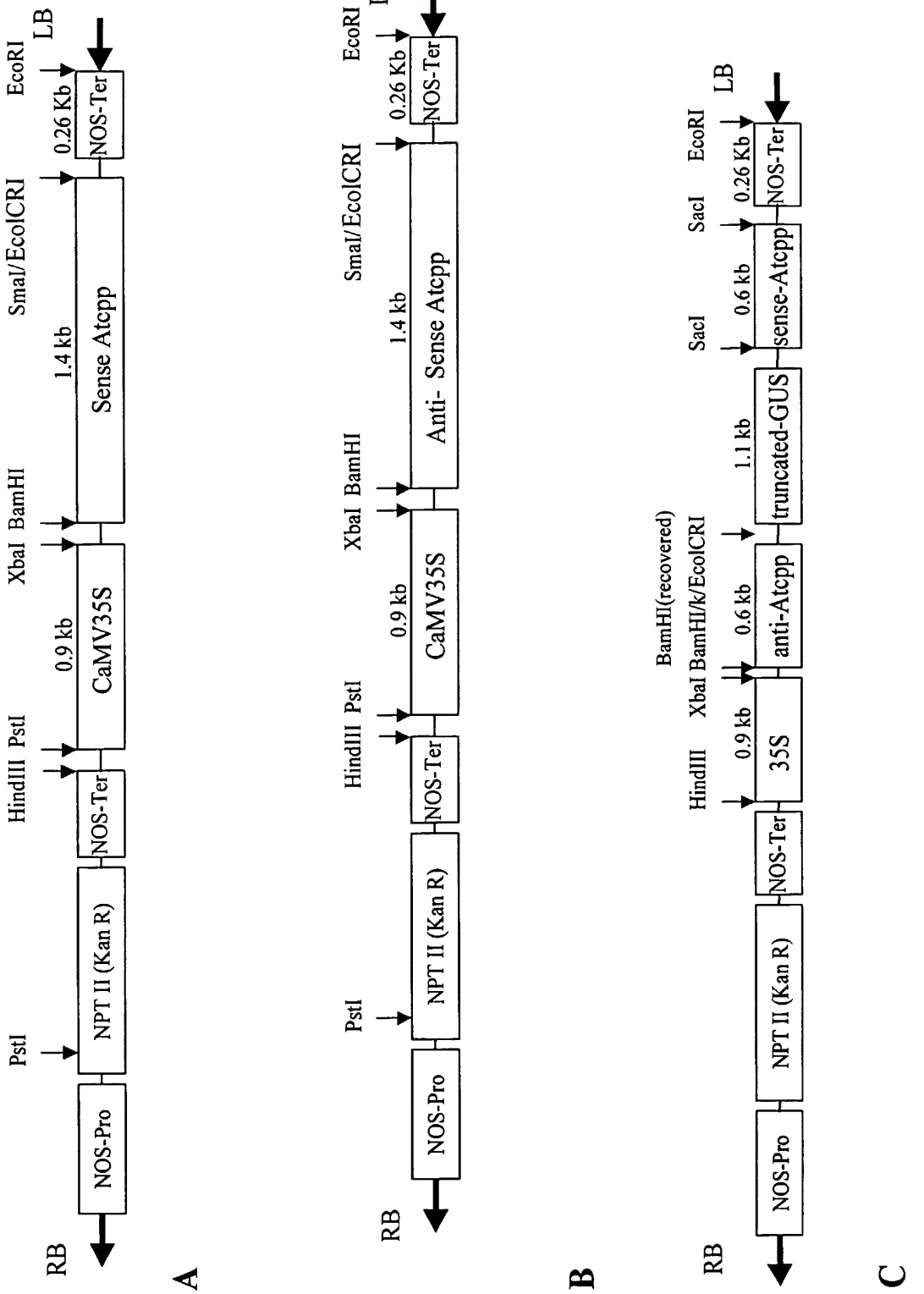

RB
NOS-Pro
PstI
NPT II (Kan R)
HindIII PstI
NOS-Ter
0.9 kb
CaMV35S
XbaI BamHI
1.4 kb
Sense Atcpp
SmaI/EcoICRI
0.26 Kb
EcoRI
NOS-Ter
LB
A
RB
NOS-Pro
PstI
NPT II (Kan R)
HindIII PstI
NOS-Ter
0.9 kb
CaMV35S
XbaI BamHI
1.4 kb
Anti- Sense Atcpp
SmaI/EcoICRI
0.26 Kb
EcoRI
NOS-Ter
LB
B
RB
NOS-Pro
HindIII
NPT II (Kan R)
NOS-Ter
0.9 kb
35S
XbaI BamHI/k/EcoICRI
BamHI(recovered)
0.6 kb
anti-Atcpp
1.1 kb
truncated-GUS
SacI
0.6 kb
sense-Atcpp
SacI EcoRI
0.26 Kb
NOS-Ter
LB
C

Figure 25.

A

Nucleic Acid	PPI-AtCPP	PPI-BnCPP	PPI-SoyCPP	BASF-AT1	BASF-AT2	BASF-Corn	BASF-Soy	AFC1	AT4g01320	AF007269
PPI-AtCPP	X									
PPI-BnCPP	92	X								
PPI-SoyCPP	76	77	X							
BASF-AT1	98	93	76	X						
BASF-AT2	99	93	76	99	X					
BASF-Corn	57	57	57	57	57	X				
BASF-Soy	72	72	93	72	72	52	X			
AFC1	99	93	77	99	99	57	72	X		
AT4g01320	99	92	70	99	99	50	64	99	X	
AF007269	97	91	10	97	97	13	8	97	97	X

B

Amino Acid	PPI-AtCPP	PPI-BnCPP	PPI-SoyCPP	BASF-AT1	BASF-AT2	BASF-Corn	BASF-Soy	AFC1	AT4g01320	AF007269
PPI-AtCPP	X									
PPI-BnCPP	94	X								
PPI-SoyCPP	83	83	X							
BASF-AT1	98	95	83	X						
BASF-AT2	99	95	83	99	X					
BASF-Corn	82	82	79	82	82	X				
BASF-Soy	83	83	99	83	83	73	X			
AFC1	98	95	83	99	99	82	83	X		
AT4g01320	95	93	82	96	96	72	76	96	X	
AF007269	98	94	82	98	99	82	82	98	100	X

Figure 26

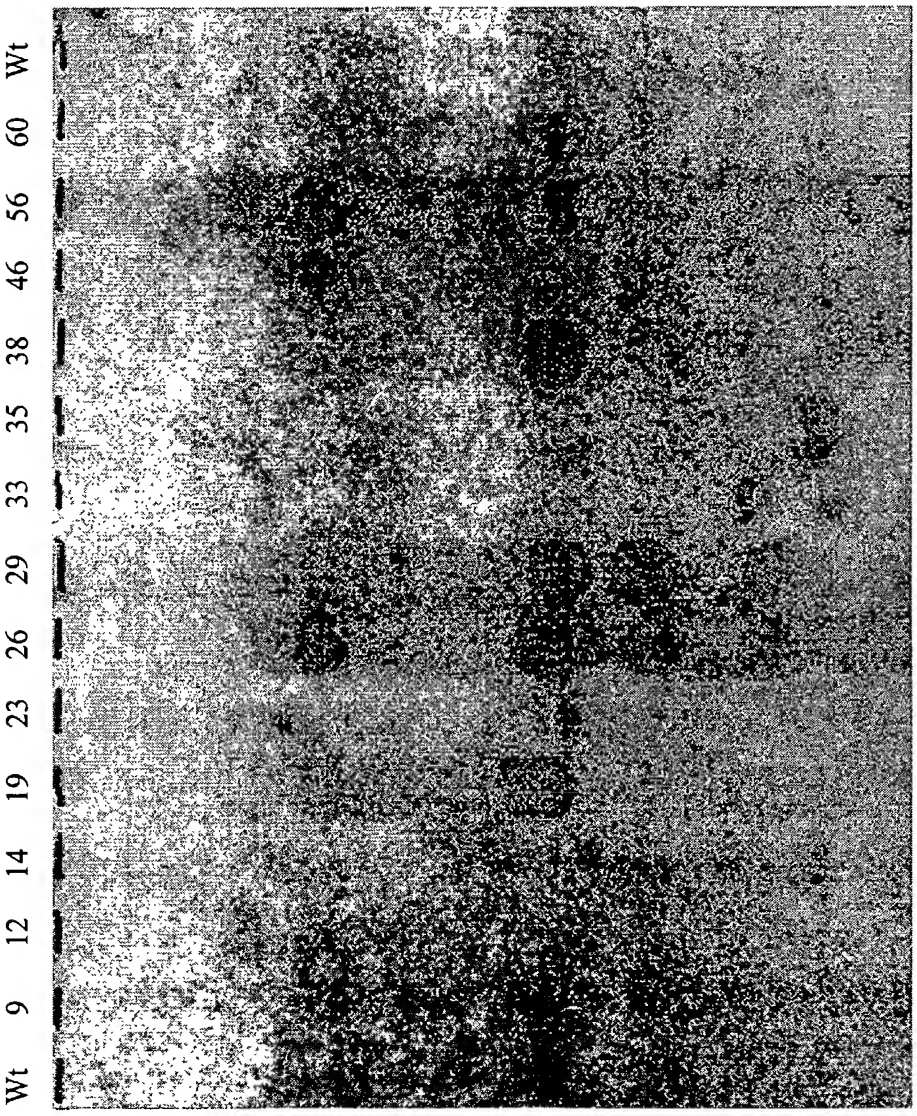


Figure 27.

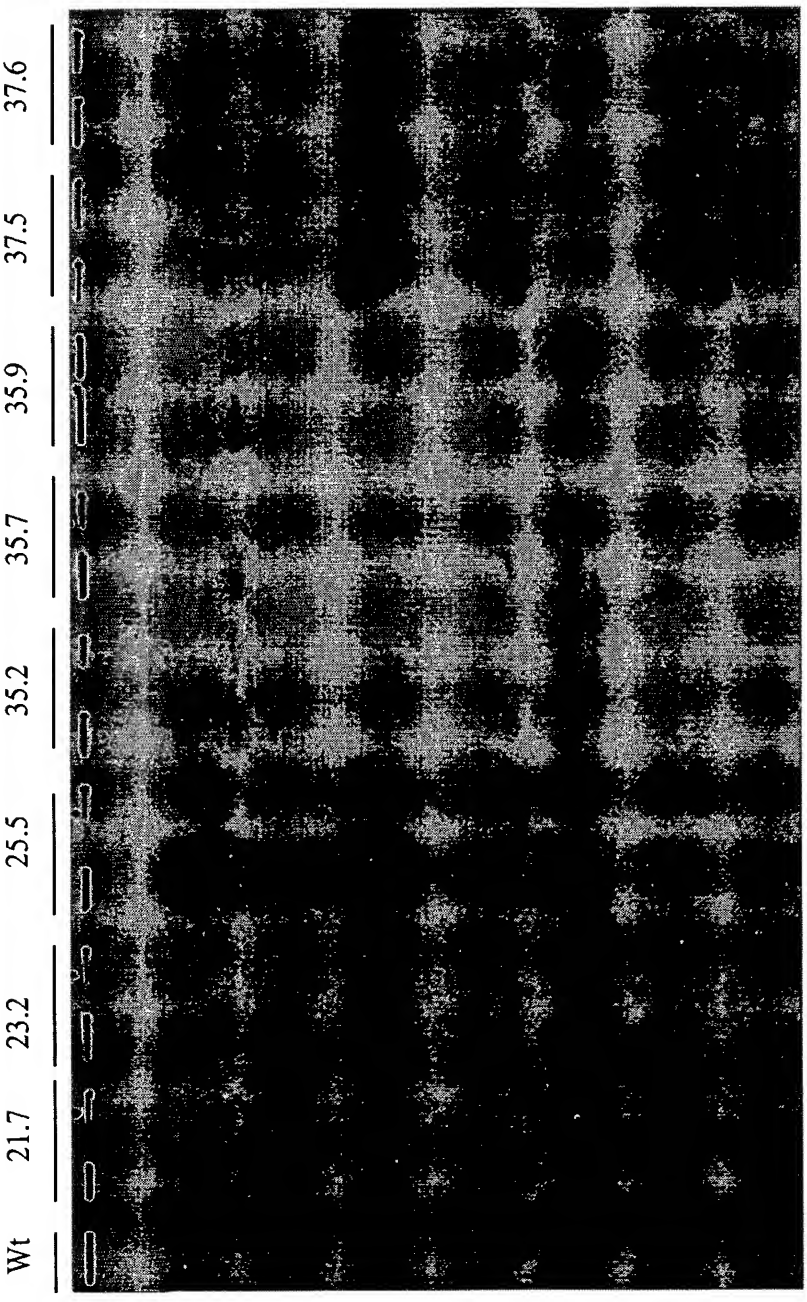


Figure 28.

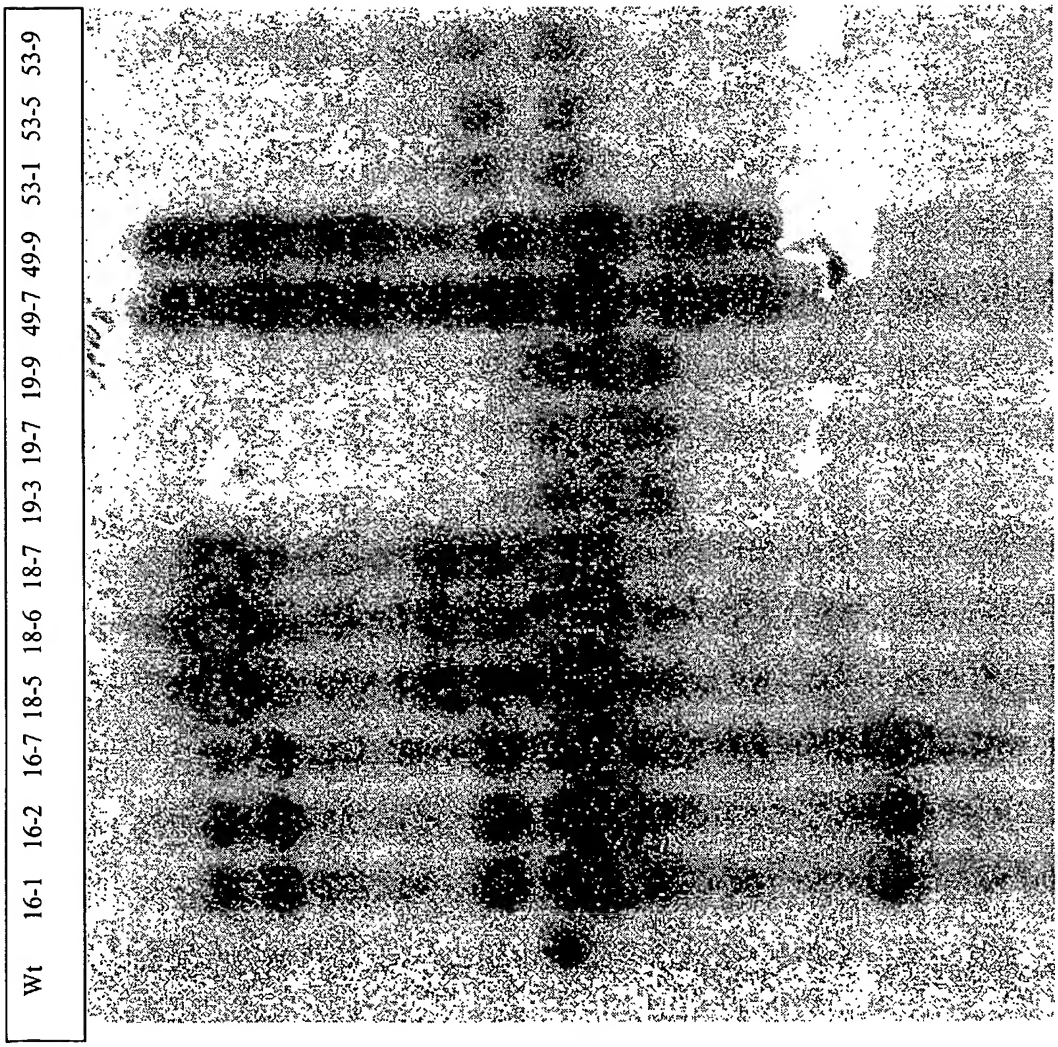


Figure 29

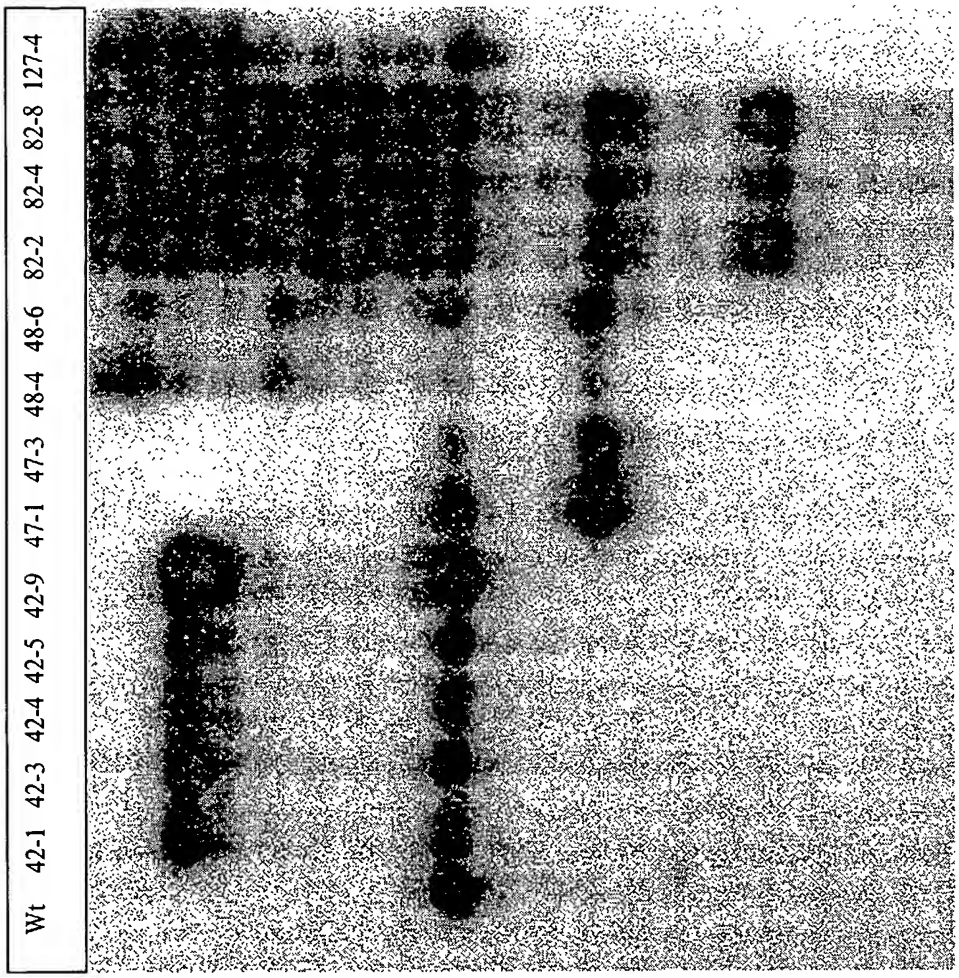


Figure 30

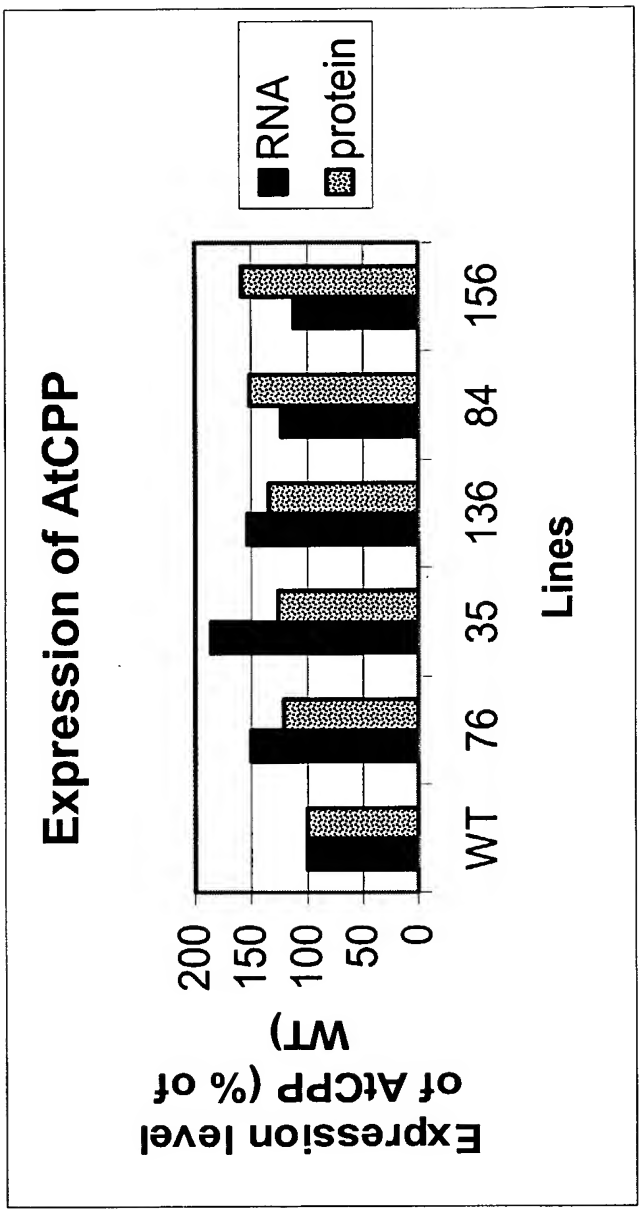

Expression of AtCPP
RNA
protein
Expression level of AtCPP (% of WT)
200
150
100
50
0
WT
76
35
136
84
156
Lines

Figure 31

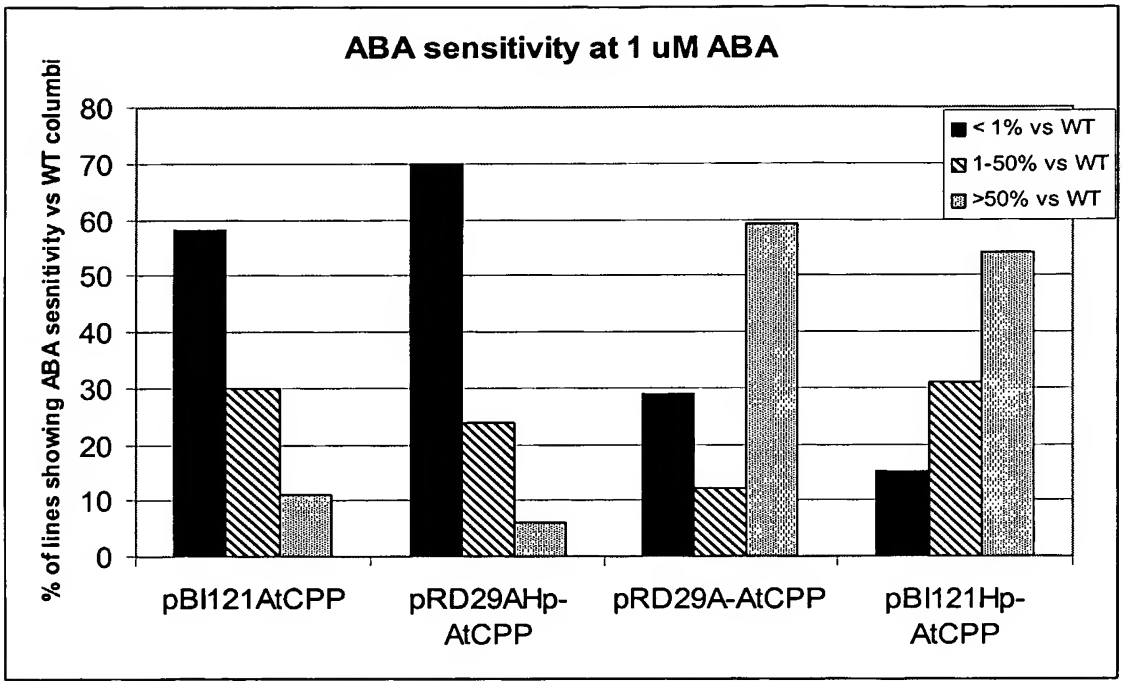

ABA sensitivity at 1 uM ABA
% of lines showing ABA sesnitivity vs WT columbi
80
70
60
50
40
30
20
10
0
< 1% vs WT
1-50% vs WT
>50% vs WT
pBI121AtCPP
pRD29AHp-AtCPP
pRD29A-AtCPP
pBI121Hp-AtCPP

Figure 32

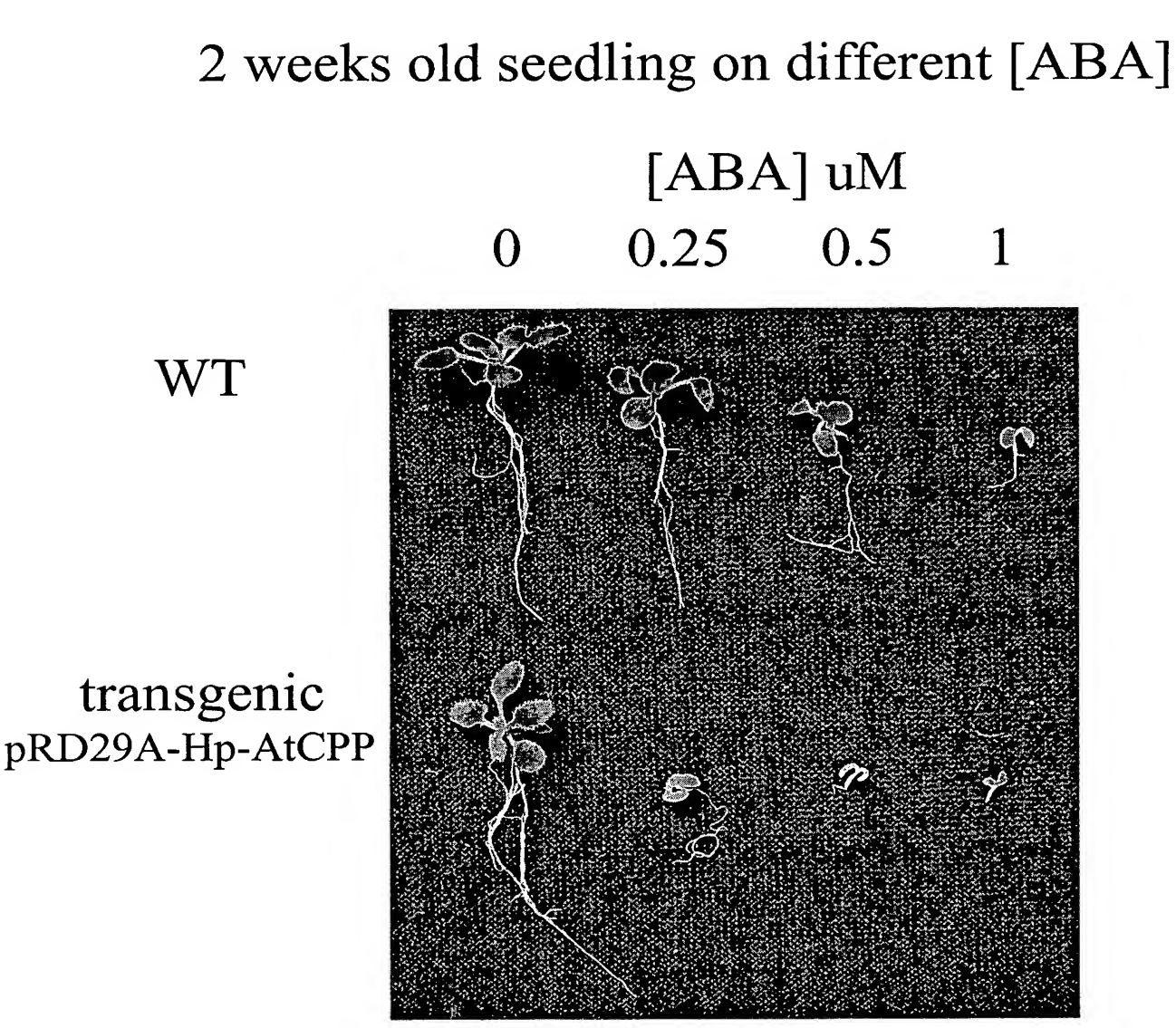

2 weeks old seedling on different [ABA]
[ABA] uM
0
0.25
0.5
1
WT
transgenic
pRD29A-Hp-AtCPP

Figure 33

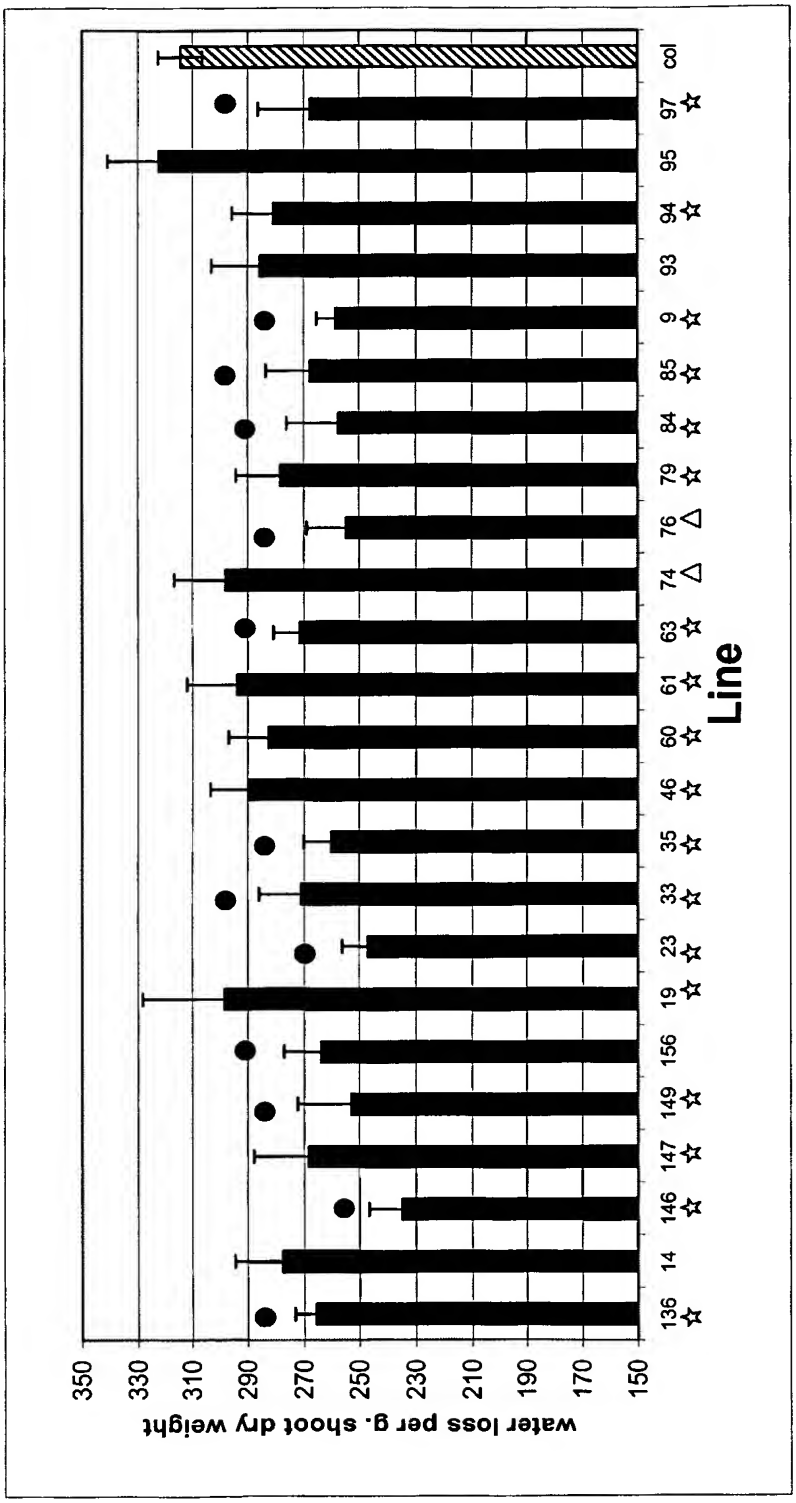

water loss per g. shoot dry weight
350
330
310
290
270
250
230
210
190
170
150
Line
136
14
146
147
149
156
19
23
33
35
46
60
61
63
74
76
79
84
85
9
93
94
95
97
col

Figure 34.

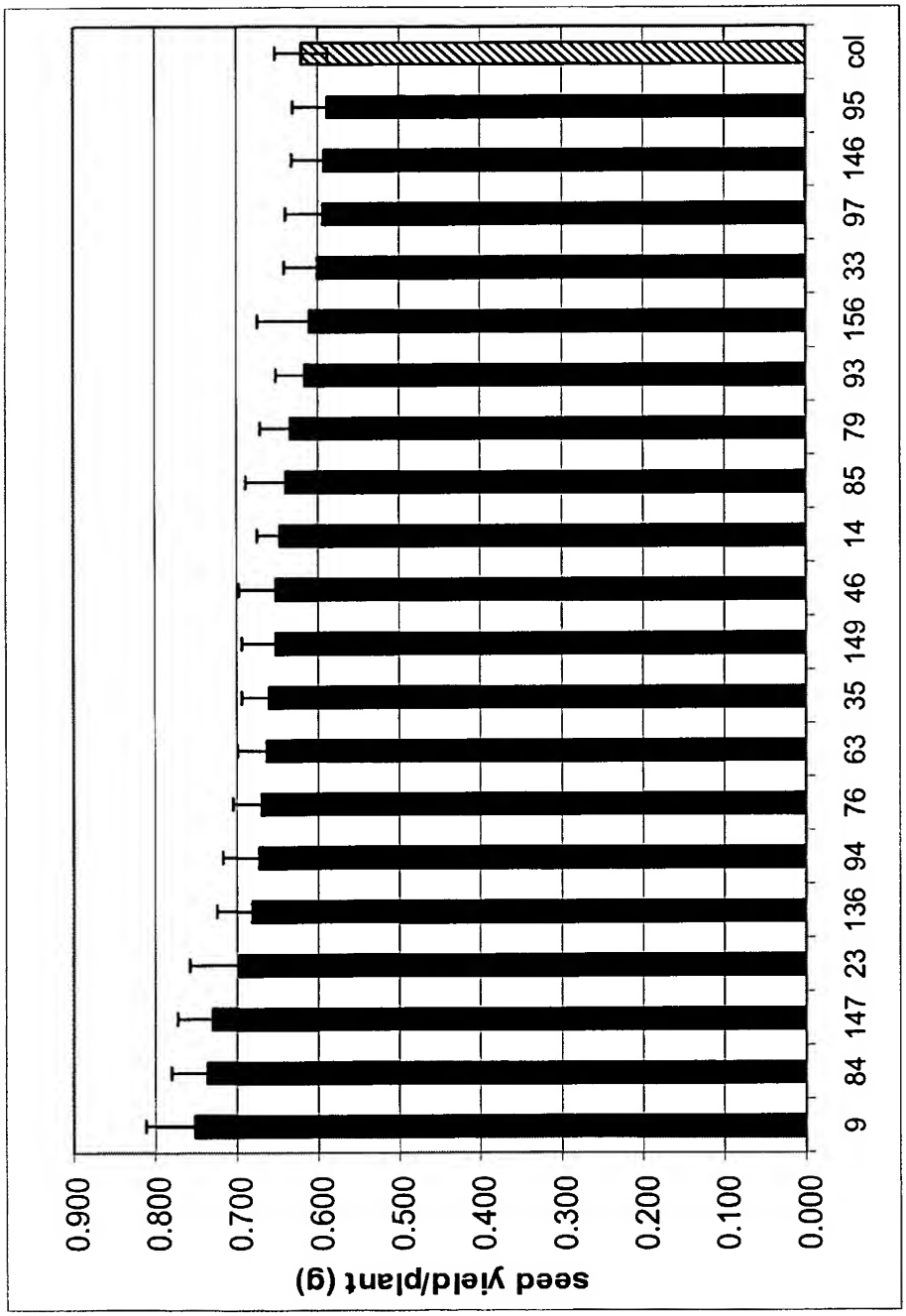

seed yield/plant (g)
0.900
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
9
84
147
23
136
94
76
63
35
149
46
14
85
79
93
156
33
97
146
95
col

Figure 35

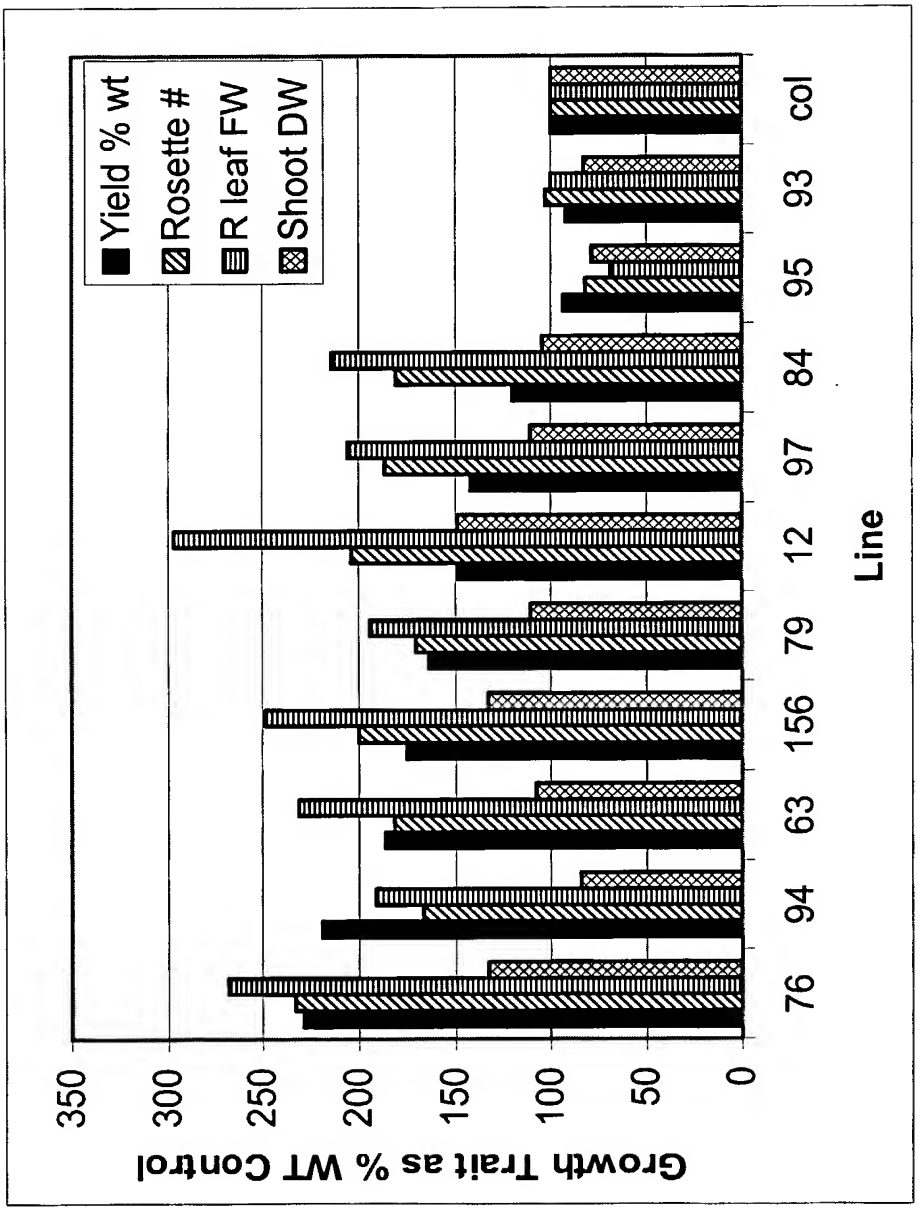

Growth Trait as % WT Control
350
300
250
200
150
100
50
0
Yield % wt
Rosette #
R leaf FW
Shoot DW
76
94
63
156
79
12
97
84
95
93
col
Line

Figure 36

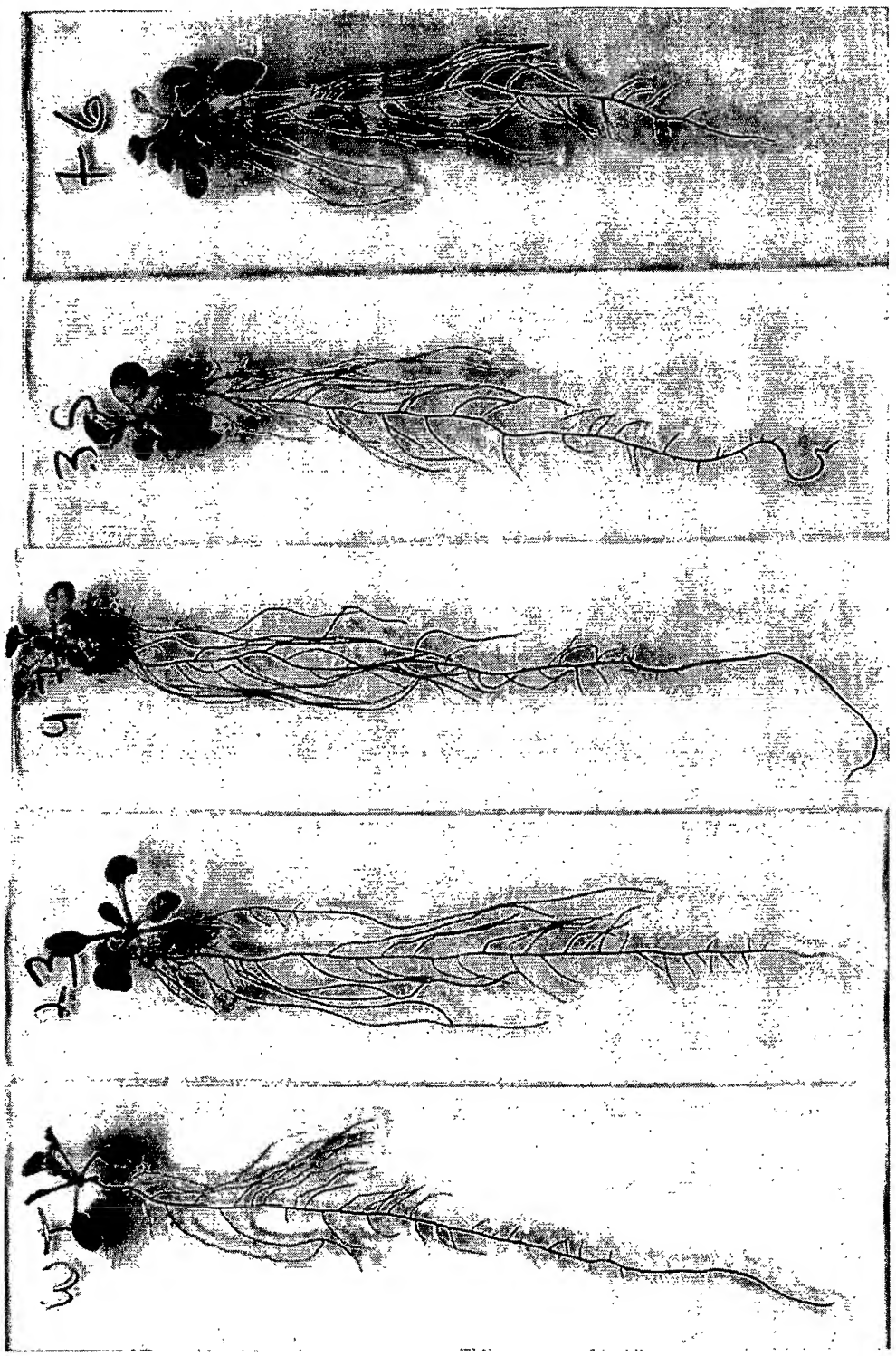


Figure 37

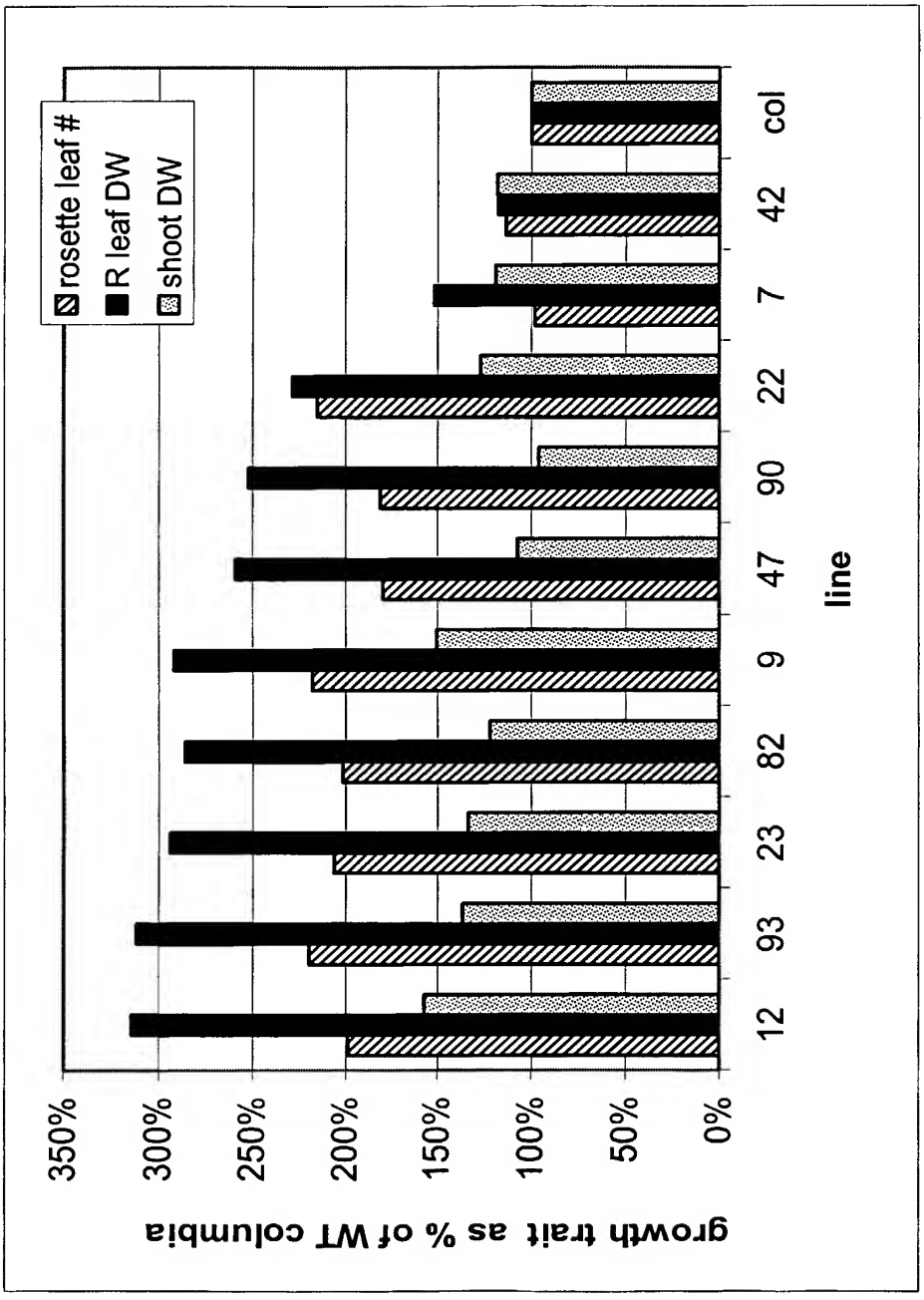

rosette leaf #
R leaf DW
shoot DW
growth trait as % of WT columbia
350%
300%
250%
200%
150%
100%
50%
0%
12
93
23
82
9
47
90
22
7
42
col
line

Figure 38

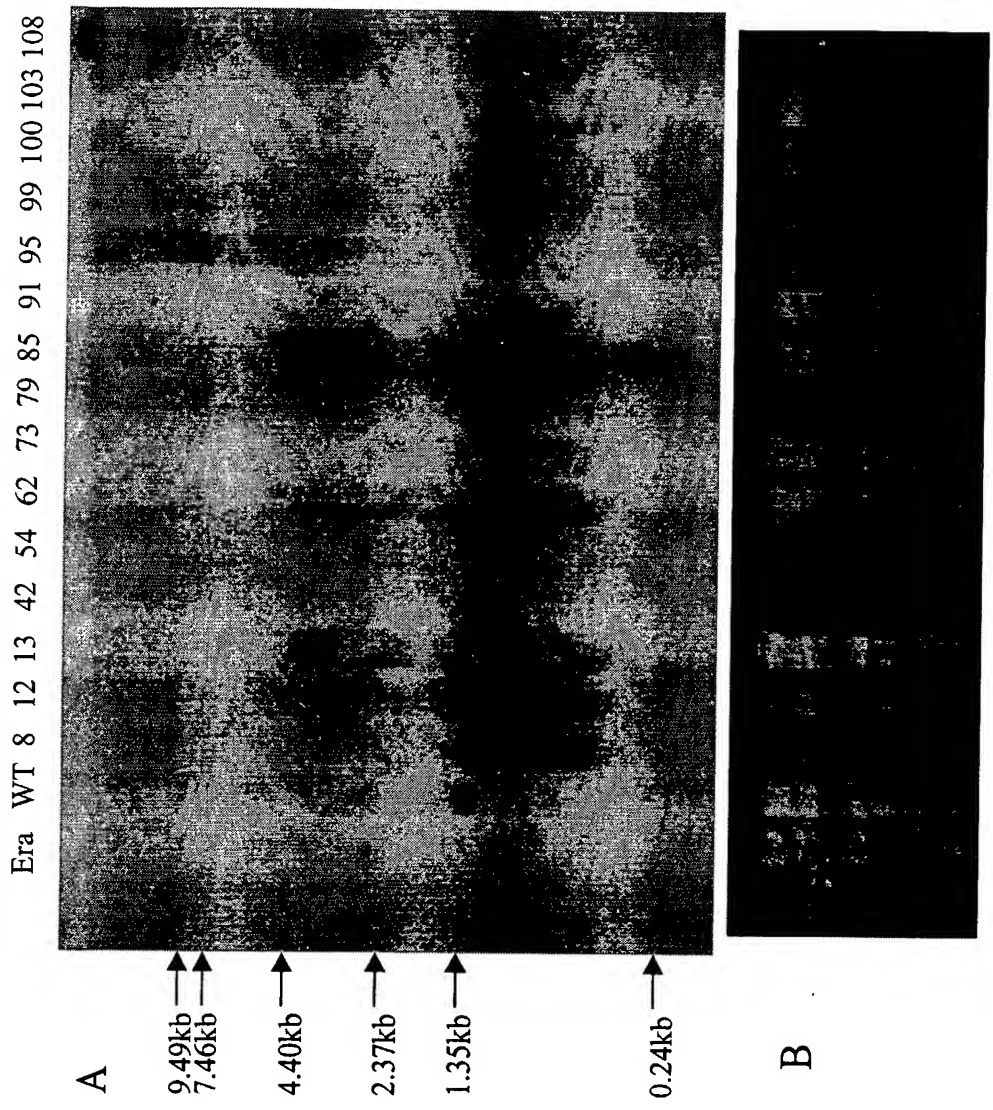


Figure 39 . Northern blot of ΔN90AtFTB arabidopsis plants

A. Northern blot probed with ΔN90AtFTB DNA probe

B. Ethidium bromide stain of agarose gel showing RNA loading per lane